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THE RESPIRATION OF BARTLETT PEARS[1]

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INTRODUCTION

In a previous report (*11*) [2] one of the writers discussed the keeping quality and the chemical composition of Bartlett pears when held at various temperatures, from the time of picking until full yellow-ripe. It was found that both sugars and acids were higher and the ripened product was of the finest eating quality when the fruit was held from the time of picking until soft-ripe at 60° to 70° F. rather than at cold-storage temperatures. It was found also that if it is desirable to hold the fruit for a longer period than is possible at the 60° to 70° F. temperature, the highest-quality product is obtained by storing at about 30° F., then removing entirely from cold storage while still firm and green-colored and allowing the fruit to become yellow and soft at the optimum temperature of 60° to 70° F. This method has invariably given a better-flavored and finer-textured ripe product than any method whereby the fruit is allowed to ripen in cold storage. No injury could be detected from cooling fruit to the very minimum above freezing and then removing it from storage and allowing it to ripen at higher temperatures.

In order to determine as nearly as possible the physiological processes concerned in the ripening of the fruit and the influence of various temperatures of storage on these processes, it was planned to measure the CO_2 given off by the fruit from the time of picking until fully mature under the different storage conditions. Determinations of the catalase content and of the concentrations of CO_2 and oxygen within the tissues were planned also.

The gaseous exchange of fruits and vegetables, both during growth and storage after growth, has attracted the attention of plant physiologists and chemists for more than a century. Unfortunately much of this work has been exceedingly fragmentary. Also very much of it has been carried on at temperatures and under other conditions entirely foreign to the commercial handling of our fruit crops. As the early literature on plant respiration and gaseous exchanges, particularly as it applies to fruits, has been quite throroughly reviewed by Bigelow, Gore, and Howard (*4*) and by Hill (*10*), it is not reviewed in this paper. Later work on respiration of fruits will be included in the discussion of results obtained in the present investigations.

PROCEDURE

One of the writers (*13*) has pointed out that there is a marked variation in the length of time that Bartlett pears from the various

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[2] Reference is made by number (italic) to "Literature cited," p. 831.

producing sections will remain firm and in good condition after removal from the tree. Fruit from warm, dry sections will remain in firm commercial condition very much longer than will that from other sections having a cooler, moister growing season. So it was planned to obtain fruit from one section which had good shipping and keeping qualities, and from another and cooler section producing a poorer-shipping Bartlett.

Bartlett pears grown in the vicinity of Sacramento, Calif., have been found to be of very good storage and shipping quality. Such fruit was obtained from an orchard near Sacramento. This variety from the cooler, more humid Santa Clara Valley has been found to be much poorer in shipping quality, often breaking down on the inside about the core region while still appearing firm on the outside. Fruit grown near Santa Clara, Calif., was obtained as representative of this type.

During previous work (*11*) it was found that a very wide variation occurs among fruits from the same tree. Fruit picked early in the commercial season requires a much longer time to become sufficiently ripe for eating but remains in prime eating condition for only a short time, while fruit more mature when removed from the tree comes to eating condition more quickly at prevailing summer temperatures but remains in firm condition much longer after becoming yellow. Consequently, it seemed advisable to use some fruit picked early (at the beginning of the fresh shipping season) and some picked much later (at the time of the last picking for cannery purposes.)

At the beginning of the season in each section, trees about 18 years old, with a full crop but not overloaded, and typical in every way, were selected. In each instance representatives of the United States Department of Agriculture picked the fruit, packed it in regular box containers, and shipped it by express to the laboratory and storage plant at Watsonville, Calif. The fruit arrived the day following picking. Immediately after arrival the fruit was redivided into three lots for storage and respiration tests. Samples were also taken for chemical analysis. The methods of sampling and analysis were the same as those outlined in a previous report (*11*).

Fruit was immediately placed in the temperature at which it was to be held, and at which respiration tests were to be run. These temperatures were as follows:

30° F.—This is the temperature previously found best for holding Bartlett pears in cold storage. The temperatures as actually obtained ranged from 28° to 32° F.

37° F.—It was possible to maintain this temperature for only about one month, and none of the fruit became ripe, but it was possible to measure the respiration of one lot at this temperature.

60° F.—A room in the cold-storage plant that was not being refrigerated was used for this temperature. Outside temperatures in this region of California fluctuate only slightly during the day, and the temperature in this room remained fairly constant, which gave satisfactory temperature conditions for the purpose.

DESCRIPTION OF RESPIRATION APPARATUS

The apparatus for the absorption of CO_2 was quite similar to that described by Gore (*7*). The fruit was held in large desiccators in most of the experiments. Large metal containers, carefully tested for air leaks, were also used in some of them, but the desiccators were

much better because the fruit could be seen and the degree of ripeness noted and there was less danger of leaks.

Carbon-dioxide-free air was obtained by passing the incoming air through U tubes containing 8 to 10 mesh soda lime. Before entering the desiccators, the air bubbled through $Ba(OH)_2$ solution to test it for traces of CO_2. Air was drawn from the bottoms of the desiccators and sucked through Reiset tubes filled with twice-normal KOH solution. These tubes were prepared in the laboratory, perforated porcelain plates being used to break up the bubbles instead of the platinum plates usually employed. The tubes were shrunk below the plate sufficiently to prevent the plate falling through, then the plate was adjusted and sealed to the tube with asphaltum of rather high melting point. Tubes were sufficiently large to contain 200 c. c. of solution, and it was found that if four finely perforated plates were used in a tube complete absorption of the CO_2 was obtained, even when the air was passed through at a rate as high as 10 liters per hour.

All connections between the desiccators and the CO_2 absorption tubes were of copper or lead tubing, the rubber couplings being reduced to a minimum and painted with asphaltum.

In all cases in which fruit in cold storage was being tested, the desiccators containing fruit were held in the refrigerated room, but the tube leading from the container to the absorption apparatus passed through the wall into a nonrefrigerated room in which the temperature was held at about 60° F.

Suction was maintained by means of a water aspirator. A mercury trap was arranged in such a way that if the water for any reason should be shut off the suction line would automatically seal itself and the liquid in the Reiset tubes would not be sucked back into the containing flasks. The rate of passage of air through the desiccators and Reiset tubes was controlled by means of screw pinchcocks at the tops of the Reiset tubes.

COLLECTION AND MEASUREMENT OF CARBON DIOXIDE

Two hundred cubic centimeters of 2 N KOH solution was put in 1-liter filter flasks, the Reiset tubes carefully sealed in the flasks, and the latter connected to the respiration chambers. All connections about and between the flasks and the desiccators containing the fruit were sealed with a mixture of eight parts asphaltum and one part parawax.

The time of the individual runs varied with the temperature and quantity of the fruit. At 30° F. the runs could be continued 14 days without changing the KOH solution. At 60°, however, in certain cases where large quantities of fruit were used, it was necessary to change the KOH solution daily.

The CO_2 was determined by the double-titration method, sulphuric acid being used, and was carried out in the same containers used for KOH during the collection of the CO_2. Phenolphthalein was used as the indicator for the KOH and K_2CO_3, and methyl orange for the $KHCO_3$. A burette with an extension tip was used during the phenolphthalein titration, and great care was exercised to keep the KOH solution thoroughly agitated and to keep the point of the burette extension well below the surface of the solution to prevent local excesses of acid and escape of CO_2.

Blank titrations were made to determine the amount of carbonate in the KOH solution. Owing to the very large amounts of CO_2

being titrated, any errors due to difficulty in detecting the phenolphthalein end point are negligible. To determine the methyl-orange end point, a standard color solution consisting of distilled water saturated with CO_2 and containing the same amount of indicator used in the regular titration was employed. The same standard was used throughout all the determinations.

The rate at which the air was passed through the respiration chambers varied with the temperature. In the cold-storage rooms it was found by calculation that about 1 liter per hour was sufficient to keep the concentration of CO_2 in the containers down to 1 per cent. At 60° F., however, air was passed through at a rate of 6 to 8 liters per hour.

RESULTS

In all of the respiration experiments at 60° F. the runs were started as quickly as possible after the fruit was picked, and continued until it was full yellow-ripe and very soft. In certain cases the experiments were continued until the fruit had passed eating condition and had become blackened inside and scalded and decayed outside.

The results of the respiration runs are presented in Tables I to XV, inclusive, and also as curves in Figures 1, 2, and 3. Table I gives the results for the Sacramento Bartletts picked July 14, early in the shipping season, and held at 60° F.

TABLE I.—*Respiration of Bartlett pears, from the Sacramento Valley, Calif., picked July 14, 1920*

Run No.	When started	Length of run	Kilogram hours	Weight of CO_2 given off	CO_2 per kilogram hour	Average temperature
		Hours		*Gm.*	*Mgm.*	° *F.*
1	July 19, 4.00 p. m.	93.5	384.0	3.311	8.62	59.0
2	July 24, 9.30 a. m.	126.25	518.4	11.076	21.37	59.0
3	July 29, 3.45 p. m.	88.5	361.4	11.578	32.04	58.0
4	Aug. 2, 2.00 p. m.	45.17	185.5	7.598	40.96	58.5
5	Aug. 4, 11.10 a. m.	50.83	208.75	10.903	52.23	60.0

Weight of fruit, beginning of run ..... 4,106.5 gm.
Weight of fruit, end of run ..... 4,068.0 gm.

CHEMICAL ANALYSIS OF CONCOMITANT LOT

	Reducing sugar	Total sugar	Acid as malic	Total dry matter
	Per cent	*Per cent*	*Per cent*	*Per cent*
Beginning of run No. 1	5.95	6.37	0.3082	17.67
End of run No. 3, firm yellow-ripe	7.32	8.03	.3551	18.07

The respiration data given in Table I are graphically shown in curve 1 of Figure 1. From the first run until the last there is a great increase in the rate of evolution of carbon dioxide, notwithstanding the fact that the temperature remained nearly constant. This fruit was full yellow-ripe during the fourth run, but when removed at the end of the fifth run it was still in firm condition throughout.

Table II and curve 2 of Figure 1 give the results from the Santa Clara Valley fruit, picked August 4, 1920. This fruit was somewhat larger than the Sacramento fruit reported in Table I, but it

was representative of the fruit about the beginning of the picking season in that region. As most of the fruit from this section goes to the canneries, the early pickings are later than those of the Sacramento section, which are intended for eastern shipment. However, the two runs are fairly comparable so far as degree of maturity at the time of picking is concerned.

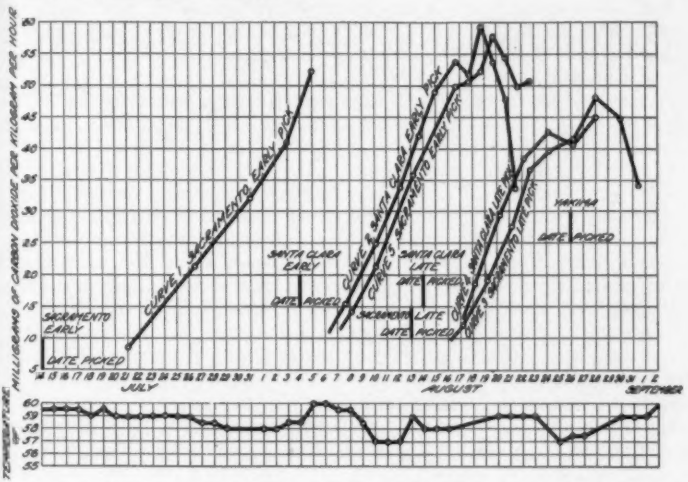

FIG. 1.—Respiration of early and late picked Sacramento and Santa Clara Valley (Calif.) Bartlett pears while in storage at about 60° F.

TABLE II.—*Respiration of Bartlett pears from the Santa Clara Valley, Calif., picked August 4, 1920*

Run No.	When started	Length of run	Kilogram hours	Weight of CO_2 given off	CO_2 per kilogram hour	Average temperature
		Hours		*Gm.*	*Mgm.*	° *F.*
1	Aug. 6, 5.15 p. m.	71.5	305.3	4.703	15.40	59
2	Aug. 9, 4.45 p. m.	47.5	202.8	5.042	24.86	57
3	Aug. 11, 4.15 p. m.	48.25	206.0	6.939	33.68	56
4	Aug. 13, 4.30 p. m.	24.5	104.6	4.395	42.02	58
5	Aug. 14, 5.00 p. m.	45.25	193.2	9.449	48.91	58
6	Aug. 16, 2.15 p. m.	27.25	116.4	6.261	53.79	59
7	Aug. 17, 5.30 p. m.	23.83	101.8	5.259	51.66	----
8	Aug. 18, 5.20 p. m.	22.67	96.8	5.731	59.21	59
9	Aug. 19, 4.00 p. m.	24.5	104.6	5.612	53.65	59
10	Aug. 20, 4.30 p. m.	22.5	96.1	4.591	47.77	59
11	Aug. 21, 3.00 p. m.	20.0	85.4	2.871	33.62	59

Weight of fruit, beginning of run ..... 4,270 gm.
Weight of fruit, end of run ..... 4,252 gm.

CHEMICAL ANALYSIS OF CONCOMITANT LOT

	Reducing sugar	Total sugar	Acid as malic	Total dry matter
	Per cent	*Per cent*	*Per cent*	*Per cent*
Beginning of run No. 1	6.60	7.27	0.1970	14.03
When full ripe, run No. 7	6.46	8.38	.1936	----

The initial rate of CO_2 evolution in the Santa Clara fruit is markedly above that of the first run of Sacramento fruit, notwithstanding the fact that a much longer time elapsed between the picking and the start of the first run in the case of the Sacramento fruit. Also, the increase is more abrupt in the case of the poorer-keeping Santa Clara fruit. Twelve days after picking the Sacramento fruit was giving off only 20 mgm. of CO_2 per kilogram hour, while after the same interval the Santa Clara fruit was giving off about 50 mgm. This will be discussed further in connection with the general relation of respiration to ripening.

By the middle of run No. 5 the Santa Clara fruit was full yellow and soft. The CO_2 evolution at this time was nearly 50 mgm. per kilogram hour. The rate continued to increase, more or less irregularly, until breaking down began, when the CO_2 output diminished rapidly. At the end of the final run fruit was badly decayed on the outside and the core region was entirely darkened and broken down.

For the later picking from the Sacramento Valley the fruit was gathered August 13. This fruit was representative of fruit picked at the extreme end of the picking season in that section, August 13 being later than fruit for eastern shipment is usually picked. This fruit was showing a marked yellow ground color below the green at the time of picking. It was in the condition which the previous season's work had shown to be best for cold-storage purposes, and which resulted in the very highest quality when the fruit was ripened out of cold storage. The respiration of this fruit, when held at approximately 60° F., is given in Table III and in curve 3 of Figure 1.

TABLE III.—*Respiration of Bartlett pears from the Sacramento Valley, Calif., picked August 13, 1920*

Run No.	When started	Length of run	Kilogram hours	Weight of CO_2 given off	CO_2 per kilogram hour	Average temperature
		Hours		*Gm.*	*Mgm.*	° *F.*
1	Aug. 16, 3.45 p. m	49.5	208.4	2.507	12.03	
2	Aug. 18, 5.15 p. m	47.25	199.0	3.797	19.08	59.0
3	Aug. 20, 4.30 p. m	44.0	185.3	5.138	27.73	59.0
4	Aug. 22, 12.30 p. m	28.5	120.0	4.382	36.52	59.0
5	Aug. 23, 5.00 p. m	46.25	194.7	7.709	39.59	58.0
6	Aug. 25, 3.15 p. m	48.75	205.3	8.497	41.39	57.5
7	Aug. 27, 4.00 p. m	43.0	181.1	8.698	48.03	59.0
8	Aug. 29, 11.00 a. m	47.33	199.3	8.901	44.66	59.0
9	Aug. 31, 10.20 a. m	27.92	117.6	4.008	34.08	59.9

Weight of fruit, beginning of run ..... 4,211 gm.
Weight of fruit, end of run ..... 4,181 gm.

CHEMICAL ANALYSIS OF CONCOMITANT LOT

	Reducing sugar	Total sugar	Acid as malic	Total dry matter
	Per cent	*Per cent*	*Per cent*	*Per cent*
Beginning of run No. 1	7.05	7.63	0.2961	16.80
Full ripe, run No. 6	7.78	9.50	.3149	16.86

For the late-pick Santa Clara Valley pears, fruit was gathered August 14. The two pickings of this fruit were made much closer together in point of time than those of the Sacramento fruit, which is in line with the commercial practice in the Santa Clara district. So far as the actual maturity of the fruit is concerned, the early-picked Sacramento lot was probably greener than the first pick from Santa Clara, while the late-pick Sacramento fruit was more nearly tree-ripe than that of Santa Clara. Table IV and curve 4 of Figure 1 give the respiration data for late-picked Santa Clara pears.

TABLE IV.—*Respiration of Bartlett pears from the Santa Clara Valley, Calif., picked August 14, 1920*

Run No.	When started	Length of run	Kilogram hours	Weight of CO_2 given off	CO_2 per kilogram hour	Average temperature
		Hours		*Gm.*	*Mgm.*	° *F.*
1	Aug. 17, 5.00 p. m.	47.67	202.0	3.736	18.51	59.0
2	Aug. 19, 4.40 p. m.	45.83	194.25	5.689	29.29	59.0
3	Aug. 21, 2.30 p. m.	50.5	214.0	8.214	38.38	59.0
4	Aug. 23, 5.00 p. m.	46.25	196.0	8.323	42.46	58.0
5	Aug. 25, 3.15 p. m.	48.75	206.6	8.419	40.75	57.5
6	Aug. 27, 4.00 p. m.	43.0	182.3	8.178	44.87	57.5

Weight of fruit, beginning of run..... 4,238.5 gm.
Weight of fruit, end of run..... 4,153.0 gm.

CHEMICAL ANALYSIS OF CONCOMITANT LOT

	Reducing sugar	Total sugar	Acid as malic	Total dry matter
	Per cent	*Per cent*	*Per cent*	*Per cent*
Beginning of run No. 1	6.88	8.19	0.1695	14.59
When full ripe, run No. 5	6.58	8.80	.1836	14.42

A comparison of the data presented in Tables III and IV, shown graphically in curves 3 and 4 of Figure 1, indicates some interesting differences between the Sacramento and the Santa Clara fruit. The late-picked Sacramento fruit was more nearly tree-ripe at the time of picking than the corresponding lot from Santa Clara. Also, the Sacramento fruit showed less of the green color and a much higher yellow ground color.

The first run in each case started just three days after picking, yet the initial run of the Sacramento fruit was only 12 mgm. per kilogram hour, while that of the Santa Clara fruit was 18.5 mgm. Again, eight days after picking (middle of run No. 3) the Sacramento fruit was giving off CO_2 at the rate of only 27 mgm. per kilogram hour, while the rate for the Santa Clara fruit was above 38 mgm. (middle of run No. 3). Thus, notwithstanding the fact that the Sacramento fruit was apparently riper at the time of picking, the Santa Clara fruit showed a higher respiration rate at any interval up to the time of full yellow color and soft-ripe condition, which is in accordance with the record of the number of days required for this fruit to become full soft-ripe, for one to two days more were required for the Sacramento than for the Santa Clara pears.

COMPARISON OF RESPIRATION IN EARLY AND LATE PICKED BARTLETT PEARS

There was a much more marked difference between the respiration rates of the early and the late picked pears from Sacramento than between those of the early and the late picked fruit from Santa Clara. This is to be expected, however, considering that there was an interval of 30 days between the two pickings of Sacramento pears, and only 10 days between the Santa Clara pickings.

The initial respiration rate was markedly lower in the early-picked fruit from both localities. Although five days elapsed before the start of the first run on the Sacramento fruit, the initial rate was only 8.62 mgm. per kilogram hour; while the initial rate on the late-picked fruit was 12.03 mgm., and only three days elapsed after picking before the run started. During run No. 2 of Table III, which was started five days after picking, the late-picked fruit gave off CO_2 at the rate of 19.08 mgm. of CO_2 per kilogram hour. Similarly, the intial runs of the Santa Clara fruit showed 15.40 mgm. for the early-picked fruit, and 18.51 mgm. for the late-picked fruit.

The speed with which the respiration rate increased was less rapid in the early than in the late picked fruit. Ten days were required for the early-picked Sacramento fruit to increase above the initial respiration rate as much as 24 mgm. per kilogram hour, while this amount of increase occurred in the late-picked fruit in less than six days. Similarly, though less marked, five days were required for the early-picked Santa Clara fruit to increase to the extent of 20 mgm., while late-picked fruit increased to the same extent in four days.

The highest rate of respiration, however, was attained in all cases on early-picked fruit. Although the early-picked Sacramento fruit was not run long enough to determine the exact maximum, it had reached a rate of 52.23 mgm. of CO_2 per kilogram hour on the last run, while the early-picked Santa Clara fruit reached 59.21 mgm. The maximums for the late-picked fruit were 48.03 mgm. for Sacramento and 44.87 mgm. for Santa Clara.

The curves for early-picked fruit, including curves 1, 2, and 5 of Figure 1, are more abrupt at their highest points than those for late-picked fruit. The respiration rate of early-picked fruit increases to a sharp maximum, then quickly falls off as the fruit breaks down, while for the late-picked fruit the approach to the maximum is more gradual. Previous observation (*11*, *13*) had shown that well-matured fruit remained in prime eating condition longer than fruit picked in more immature condition. The fact that early-picked fruit respires faster when it is in prime eating condition than does late-picked fruit from the same trees, offers a partial explanation for the rapid breaking down of such fruit after reaching prime eating condition.

Table V and curve 1 of Figure 2 present the respiration data for one lot of pears of mid-season pick from the Yakima district in Washington. This fruit was four days en route at unknown temperatures, and five days elapsed between the time of picking and the start of the respiration experiment.

TABLE V.—*Respiration of Bartlett pears from the Yakima Valley, Wash., picked August 26, 1920*

Run No.	When started	Length of run	Kilogram hours	Weight of CO_2 given off	CO_2 per kilogram hour	Average temperature
		Hours		*Gm.*	*Mgm.*	*° F.*
1	Aug. 31, 12.00 m	52. 5	229. 8	5. 405	23. 52	59. 0
2	Sept. 2, 4.30 p. m	47. 5	208. 0	7. 523	36. 15	60. 0
3	Sept. 4, 4.00 p. m	43. 33	189. 7	8. 369	44. 12	59. 5
4	Sept. 6, 11.20 a. m	28. 92	126. 6	6. 217	49. 11	59. 0
5	Sept. 7, 4.15 p. m	40. 33	176. 6	8. 898	50. 395	58. 0
6	Sept. 9, 8.35 a. m	31. 92	139. 7	6. 904	49. 40	57. 0
7	Sept. 10, 4.30 p. m	23. 67	103. 6	4. 951	47. 78	56. 5
8	Sept. 11, 4.10 p. m	43. 0	188. 3	8. 151	43. 30	56. 0
9	Sept. 13, 11.10 a. m	51. 33	224. 7	8. 876	39. 49	55. 0

Weight of fruit, beginning of run ..... 4,378 gm.
Weight of fruit, end of run ..... 4,337 gm.

CHEMICAL ANALYSIS OF CONCOMITANT LOT

	Reducing sugar	Total sugar	Acid as malic	Total dry matter
	Per cent	*Per cent*	*Per cent*	*Per cent*
Beginning of run No. 1	6. 82	7. 57	0. 4556	16. 50
When full ripe, run No. 6	7. 36	8. 73	. 4770	----------

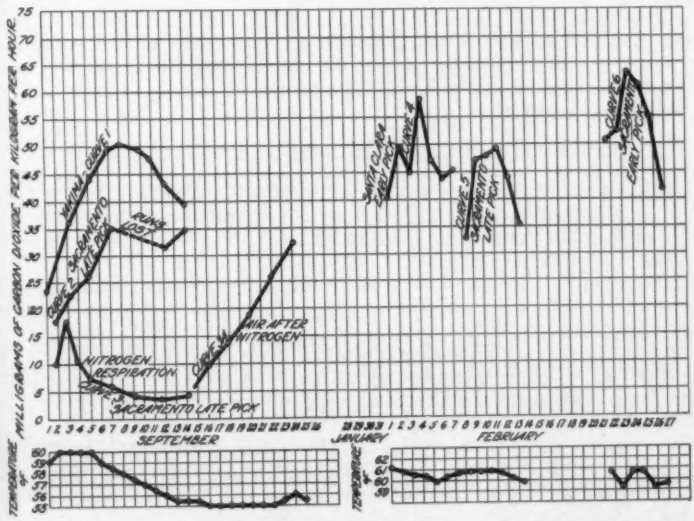

FIG. 2.—Respiration of Bartlett pears in an atmosphere of nitrogen, and respiration at 60° F. after holding in cold storage

From a study of the data it will be noted that the respiration rate of this fruit was similar to that of the fruit from Santa Clara. Six days after picking the respiration rate was about 23.5 mgm. of CO_2 per kilogram hour, almost exactly the same as the early-picked Santa Clara pears. The highest rate is intermediate between the two pickings of Santa Clara fruit, and the rate of increase in CO_2

evolution is very similar to that of the Santa Clara pears. This is especially interesting in view of the fact that in the commercial handling of the fruit from these two sections a marked similarity in the fruit has been found. Fruit from either district is shipped to distant markets only with difficulty, and has a marked tendency to soften and discolor about the core before showing deterioration on the surface.

From a study of all the data presented, it is evident that there is a marked correlation between the respiratory activity of Bartlett pears (as measured by the amount of carbon dioxide given off) and their ripening reaction. Fruit from all sections studied shows an increase in the rate of respiration from the time of picking until the fruit is soft and beginning to break down. As severe deterioration sets in, the respiration rate decreases.

The early-picked fruit respires at a much slower rate when first picked, and this rate increases more slowly than is the case with late-picked fruit. Also, the initial respiration rate of the better-keeping fruit—in this instance the Sacramento pears—was much lower than that of the fruit with poorer-keeping qualities. The respiration rate also increases faster in the poorer-keeping pears. The length of time required for the fruit to reach edible condition after picking apparently varies inversely as the respiration rate—the slower the CO_2 evolution, the longer the time required for the fruit to ripen. Late-picked fruit with a high initial respiration rate which rapidly increases after picking, comes to edible condition quickly, but the less rapid evolution of CO_2 during the ripe period is apparently associated with the longer period after ripening before breaking down begins.

RESPIRATION OF PEARS AT COLD-STORAGE TEMPERATURES

Results of earlier storage experiments with Bartlett pears (*11*) had indicated that about 30° F. was the temperature that gave the best results with pears that were to be held for a longer time than is possible in common storage. Consequently, it was planned to test the respiration of the fruit when held at this temperature throughout a long period, and also the effect of removing fruit from this temperature into warmer conditions.

Only early-picked fruit was used in the tests at 30° F. Two separate lots of Sacramento pears and one lot of early-picked Santa Clara fruit were tested. The fruit used was similar in every way to that used in the tests run at the higher temperatures.

The temperature in the room in which this fruit was held varied from 32° to 28° F. A continuous thermographic record was obtained, and it showed the room to have been at about 30° during most of the period. The two tests on early-picked Sacramento fruit are designated as A and B, and the data are presented in Tables VI and VII; and the runs on early-picked Santa Clara fruit are recorded in Table VIII. These same data are recorded graphically as curves 2, 3 and 4 of Figure 3.

During September some of the runs were lost, owing to the temperature dropping to 28° F. and freezing the baryta water connected into the system between the soda-lime tubes and the fruit containers. The heavy suction that soon developed on the apparatus caused the

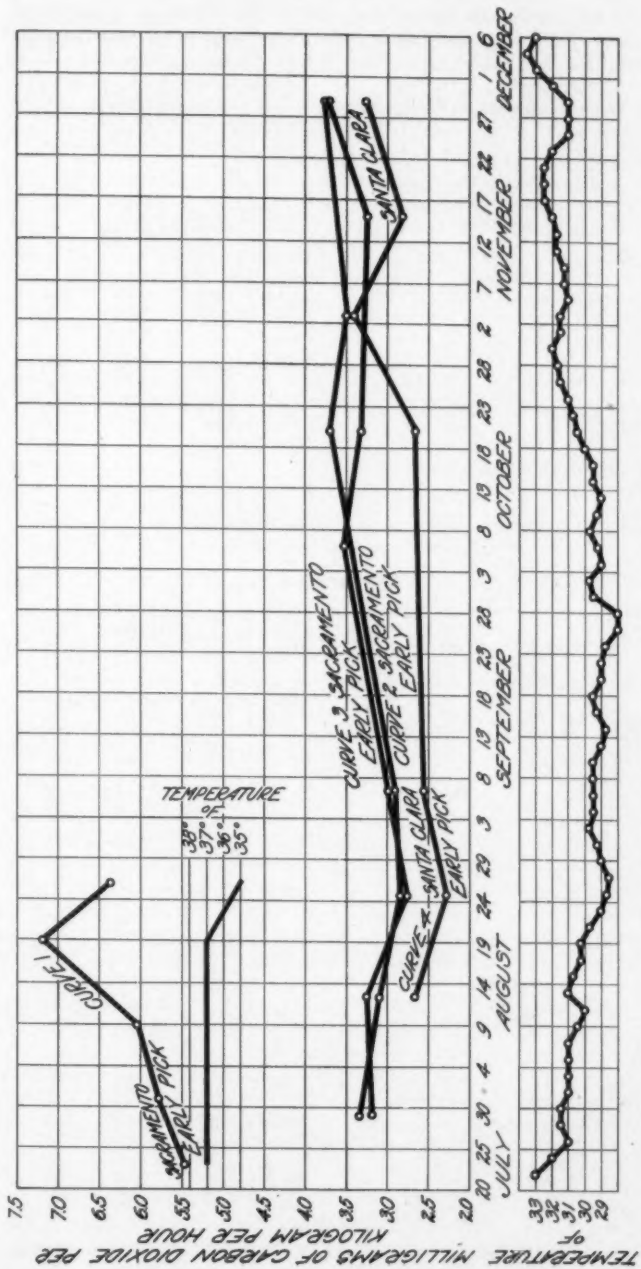

FIG. 3.—Respiration of Bartlett pears held in cold storage at 29° to 33° F.

collapse of the flasks containing the KOH solution. However, the data obtained from runs made before and after this time are entirely dependable.

TABLE VI.—*Respiration of Bartlett pears from the Sacramento Valley, Calif., picked July 14, 1920, and held in storage at 30° F.*

EXPERIMENT A

Run No.	When started	Length of run	Kilogram hours	Weight of CO_2 given off	CO_2 per kilogram hour	Average temperature
		Hours		*Gm.*	*Mgm.*	° *F.*
1	July 20, 2.00 p. m.	429.67	3,222.9	10.256	3.18	31.6
2	Aug. 7, 11.40 a. m.	268.42	2,013.4	6.538	3.24	30.5
3	Aug. 18, 4.05 p. m.	312.5	2,344.0	6.478	2.76	29.0
4	Aug. 31, 4.35 p. m.	330.5	2,479.1	7.395	2.98	29.4
5[a]						28.5
6[a]	Sept. 29, 10.45 a. m.	340.75	2,556.0	8.996	3.52	29.3
7	Oct. 13, 3.30 p. m.	362.0	2,715.4	8.988	3.31	30.7
8[a]						31.5
9	Nov. 9, 3.15 p. m.	308.0	2,310.3	7.487	3.24	32.3
10	Nov. 22, 11.15 a. m.	337.5	2,531.6	9.448	3.73	33.0

Weight of fruit at beginning of run..... 7,502 gm.

[a] Run lost.

TABLE VII.—*Respiration of Bartlett pears from the Sacramento Valley, Calif., picked July 14, 1920, and held in storage at 30° F.*

EXPERIMENT B

Run No.	When started	Length of run	Kilogram hours	Weight of CO_2 given off	CO_2 per kilogram hour	Average temperature
		Hours		*Gm.*	*Mgm.*	° *F.*
1	July 20, 2.00 p. m.	432.25	3,189.7	10.648	3.33	31.6
2	Aug. 7, 2.15 p. m.	265.83	1,961.7	6.084	3.10	30.5
3	Aug. 18, 4.05 p. m.	312.5	2,306.1	6.555	2.84	29.0
4	Aug. 31, 4.35 p. m.	330.0	2,435.2	7.096	2.91	29.4
5[a]						
6[a]						
7	Oct. 13, 3.45 p. m.	361.5	2,667.7	9.852	3.69	30.7
8	Oct. 28, 5.15 p. m.	285.75	2,108.7	7.345	3.48	31.5
9[a]						
10	Nov. 22, 11.00 a. m.	337.75	2,492.4	9.379	3.76	33.0

Weight of fruit at beginning of run..... 7,379.5 gm.

[a] Run lost.

TABLE VIII.—*Respiration of Bartlett pears from the Santa Clara Valley, Calif., picked August 4, 1920, and held in storage at 30° F.*

Run No.	When started	Length of run	Kilogram hours	Weight of CO_2 given off	CO_2 per kilogram hour	Average temperature
		Hours		*Gm.*	*Mgm.*	° *F.*
1	Aug. 7, 4.00 p. m.	264.0	2,484.2	6.615	2.66	30.5
2	Aug. 18, 4.00 p. m.	313.0	2,945.3	6.737	2.29	29.0
3	Aug. 31, 5.00 p. m.	329.67	3,102.2	7.949	2.56	29.4
4[a]						
5[a]						
6	Oct. 13, 11.30 a. m.	366.25	3,446.4	9.179	2.66	30.7
7	Oct. 28, 5.45 p. m.	285.5	2,686.6	9.190	3.42	31.5
8	Nov. 9, 3.15 p. m.	308.0	2,898.3	8.172	2.82	32.3
9	Nov. 22, 11.15 a. m.	337.5	3,175.9	10.384	3.27	33.0

Weight of fruit at beginning of run..... 9,410 gm.

[a] Run lost.

It is at once apparent that the respiration of the fruit at 30° F. markedly differs from that at 60°. Although these tests were continuous for longer than four months, there was no appreciable increase in the rate of CO_2 evolution from the first runs until the last. The slightly increased rates during the last run were due to the higher temperature.

Of much interest also is the fact that the respiration rate of the Santa Clara fruit was almost continuously lower than that of the Sacramento fruit. For only one run did the Santa Clara fruit approach the Sacramento pears in rate of respiration, and the increase during that run was so sharp as to suggest a leak in the apparatus. This is in marked contrast to the results obtained with the fruit at 60° F.

RESPIRATION OF PEARS AT 37° F.

Only one series of runs was made with fruit at 37° F., and that was for a relatively short period. This temperature was not available for longer than about one month. The respiration of the early-picked Sacramento fruit was determined during this period, however, and the results are given in Table IX and in curve 1 of Figure 2.

TABLE IX.—*Respiration of Bartlett pears from the Sacramento Valley, Calif., picked July 14, 1921, and held in storage at 37° F.*

Run No.	When started	Length of run	Kilogram hours	Weight of CO_2 given off	CO_2 per kilogram hour	Average temperature
		Hours		*Gm.*	*Mgm.*	° *F.*
1	July 19, 4.00 p. m.	186.25	846.5	4.629	5.48	37
2	July 27, 10.15 a. m.	193.08	877.7	5.081	5.78	37
3	Aug. 4, 11.20 a. m.	221.5	1,006.9	6.322	6.27	37
4	Aug. 13, 4.50 p. m.	189.2	859.9	6.174	7.18	37
5	Aug. 21, 2.00 p. m.	241.5	1,097.9	6.988	6.36	35

Weight of fruit at beginning of run ..... 4,545 gm.

At 37° F. there is a slight but well-defined increase in the amount of CO_2 given off from week to week. In only the last run did this fail to hold true; during that run the temperature was distinctly lower. During the initial run at this temperature, nearly 5.5 mgm. of carbon dioxide per kilogram-hour were evolved. The initial run at 60° C. was only 8.62 mgm. for this same lot. Thus the respiration rate after a rise in temperature of about 22° F. (or 12° C.) was only about 1.5 times the rate at the lower temperature, which is a very much smaller increase than Gore (*7*) found as an average for fruits in general, his conclusion being that the average increase in respiration rate per 10° C. rise in temperature is about 2.38 times.

If we assume the maximum respiration rate for fruit held at 60° F., however, the increase is 8 to 9 times per 12° C. temperature increase; while from 30° to 60° F. (a difference of 17° C.), the increase, assuming the maximum rate at 60°, is nearly 20 times. It is apparent that a factor other than temperature—namely, degree of maturity of the fruit—very largely determines the rate of CO_2 output in Bartlett pears, particularly at the higher temperatures.

EFFECT ON RESPIRATION RATE OF REMOVING PEARS FROM 30° F. TEMPERATURE

The opinion is widely current in commercial circles that fruit in general breaks down rapidly following removal from cold storage. It was planned to determine whether the ripening processes, as measured by CO_2 given off, are accelerated or retarded when pears are removed from cold storage. Both early and late picked Bartlett pears were held in storage at 30° F. for varying lengths of time, and then removed to 60° for respiration tests. In this way very definite information has been obtained on the effect of storage at very low temperatures on the subsequent behavior of the fruit.

The data on these experiments are presented in curve 5 of Figure 1; and curves 4, 5, and 6 of Figure 2; and in Tables X to XV, inclusive. The early-picked Sacramento pears, from which the respiration data given in Table X were obtained, were first held at 30° F. for 22 days, and their respiration at that temperature determined. They were then transferred to 60° without being removed from the container, and measuring of respiration was begun as soon as the fruit warmed to 60° F.

TABLE X.—*Respiration of Bartlett pears from the Sacramento Valley, Calif., picked July 14, and held in storage for 22 days at 30° and then transferred to 60° F.*

Run No.	When started	Length of run	Kilogram hours	Weight of CO_2 given off	CO_2 per kilogram hour	Average temperature
		Hours		*Gm.*	*Mgm.*	° *F.*
1	July 20, 2.00 p. m.	429.5	3,325.2	10.415	3.13	31.6
1A	Aug. 7, 4.40 p. m.	47.83	370.3	5.248	14.17	59.0
2A	Aug. 9, 4.30 p. m.	48.25	373.5	7.935	21.24	57.0
3A	Aug. 11, 4.45 p. m.	47.58	(a)			
4A	Aug. 13, 4.20 p. m.	24.67	190.8	6.834	35.82	58.0
5A	Aug. 14, 5.00 p. m.	42.5	329.0	13.685	41.59	58.0
6A	Aug. 16, 11.30 a. m.	30.0	232.2	11.564	49.80	59.0
7A	Aug. 17, 5.30 p. m.	24.0	185.8	9.379	50.48	
8A	Aug. 18, 5.30 p. m.	22.5	174.2	9.091	52.19	59.0
9A	Aug. 19, 4.00 p. m.	24.17	187.1	10.826	57.86	59.0
10A	Aug. 20, 4.10 p. m.	22.83	176.7	9.599	54.32	59.0
11A	Aug. 21, 3.00 p. m.	21.25	164.5	8.185	49.76	59.0
12A	Aug. 22, 12.15 p. m.	22.75	176.1	8.924	50.68	59.0

Weight of fruit at beginning of run..... 7,742 gm.
Weight of fruit at end of run..... 7,668 gm.

(a) Lost.

The fruit used for the respiration measurements recorded in Table X was exactly similar at the time of picking to that used in the tests reported in Table I. When removed from cold storage there was no immediate acceleration over fruit not held in cold storage, other than the increase which would be accounted for by the slightly riper condition of the cold-storage fruit. The initial run showed 14.17 mgm. of CO_2 per kilogram-hour after removal from cold storage, as compared with 8.62 mgm. (Table I) on fruit that was not held in cold storage.

It is interesting to calculate the total amount of CO_2 evolved per kilogram of fruit before the two lots of early-picked Sacramento pears attained a respiration rate of 21 mgm. per kilogram-hour. The lot held in cold storage remained for 22 days at slightly under 32° F., and required 3 days after removal to 60° to reach this rate, while the

lot held at 60° required longer than 12 days after picking before this rate was attained. From the established respiration rates, about 2,900 mgm. of CO_2 had been evolved per kilogram of fruit before the pears held in cold storage and then transferred to 60° attained a respiration rate of 21 mgm. of CO_2 per hour. Estimating the rate for fruit held at 60° prior to the start of the first run at 6 mgm. per kilogram-hour, this fruit evolved about 2,500 mgm. CO_2 per kilogram. After reaching this respiration rate the rate on the two lots increased at almost the same speed, and 10 days later each lot had reached a rate of between 50 and 60 mgm. per kilogram-hour. This is strong evidence that the ripening processes of Bartlett pears are intimately linked with the production of CO_2 by the fruit. Cold-storage temperatures tend to check these processes, but never entirely stop them. The time that the fruit will keep after removal from cold storage, as compared with fruit never in cold storage, is reduced just to the extent that these processes have gone on during cold storage. The lower the temperature of the storage, the more slowly the ripening processes go on. For example, the rate at 37° F., as measured by CO_2 output, is fully double that at 30° F., and the possible length of time for holding the fruit is correspondingly reduced.

This relation of cold-storage temperatures to the reaction of the fruit following removal from cold storage is further indicated by the results from leaving pears in cold storage through an extremely long season and then moving them to higher temperatures and running respiration tests. Early-picked Santa Clara fruit held at temperatures of 28° to 33° F. from the time of picking, August 4, 1920, until January 30, 1921, was removed on the latter date and a respiration test made at 60°. Late-picked Sacramento pears held in storage until February 8, and early-picked Sacramento pears held until February 21, were also tested for CO_2 output at 60° F. These data are presented in Tables XI, XII, and XIII, and in curves 4, 5, and 6 of Figure 2.

TABLE XI.—*Early-picked Bartlett pears from the Santa Clara Valley, Calif., held in storage at 28° to 33° F. from August 4, 1920, to January 31, 1921, respiration at 60°*

Run No.	When started	Length of run	Kilogram hours	Weight of CO_2 given off	CO_2 per kilogram hour	Average temperature
		Hours		*Gm.*	*Mgm.*	° *F.*
1	Jan. 31, 8.00 p. m.	37.5	168.4	6.796	40.35	61.2
2	Feb. 2, 9.30 a. m.	24.0	107.8	5.311	49.28	60.8
3	Feb. 3, 9.30 a. m.	24.0	107.8	4.852	45.02	60.4
4	Feb. 4, 9.30 a. m.	24.0	107.8	6.272	58.19	60.4
5	Feb. 5, 9.30 a. m.	25.0	112.3	5.287	47.08	59.9
6	Feb. 6, 10.30 a. m.	23.0	103.3	4.534	43.89	60.4
7	Feb. 7, 9.30 a. m.	23.5	105.5	4.781	45.30	60.6

Weight of fruit at beginning of run ..... 4,491 gm.
Weight of fruit at end of run ..... 4,452 gm.

TABLE XII.—*Late-picked Bartlett pears from the Sacramento Valley, Calif., held in storage at 28° to 33° F. from August 14, 1920, until February 8, 1921, respiration at 60°*

Run No.	When started	Length of run	Kilogram hours	Weight of CO_2 given off	CO_2 per kilogram hour	Average temperature
		Hours		*Gm.*	*Mgm.*	° *F.*
1	Feb. 8, 10.45 a. m.	23.5	101.75	3.338	32.80	60.8
2	Feb. 9, 10.15 a. m.	24.5	106.1	5.001	47.14	60.8
3	Feb. 10, 10.45 a. m.	23.0	99.59	4.759	47.79	61.0
4	Feb. 11, 9.45 a. m.	25.0	108.25	5.305	49.01	60.8
5	Feb. 12, 10.45 a. m.	25.5	110.4	4.841	43.84	60.2
6	Feb. 13, 12.15 p. m.	22.5	97.4	3.451	35.42	59.7

Weight of fruit at beginning of run ..... 4,330 gm.
Weight of fruit at end of run ..... 4,315 gm.

TABLE XIII.—*Early-picked Bartlett pears from the Sacramento Valley, Calif., held in storage at 28° to 33° F. from July 14, 1920, to February 21, 1921, respiration at 60°*

Run No.	When started	Length of run	Kilogram hours	Weight of CO_2 given off	CO_2 per kilogram hour	Average temperature
		Hours		*Gm.*	*Mgm.*	° *F.*
1	Feb. 21, 3.20 p. m.	22.67	97.7	4.943	50.60	60.8
2	Feb. 22, 2.00 p. m.	26.83	115.6	6.037	52.21	59.4
3	Feb. 23, 4.50 p. m.	23.83	102.7	6.496	63.26	60.4
4	Feb. 24, 4.40 p. m.	24.67	106.3	6.467	60.83	60.4
5	Feb. 25, 5.20 p. m.	23.0	99.1	5.427	54.75	59.4
6	Feb. 26, 4.20 p. m.	30.42	131.1	5.489	41.87	59.7

Weight of fruit at beginning of run ..... 4,309.5 gm.

The fruit in all the above lots was almost entirely yellow, though firm, at the time of its removal from cold storage. Although it had been impossible to perform respiration experiments at the lower temperature up to the time of removing the pears from cold storage, the fact that there had been no increase in the respiration rate after four months in storage seems sufficient evidence for assuming that there was no acceleration during the whole of the cold-storage run. Nevertheless, all these lots started off at a respiration rate not far from the maximum. The respiration rate in the late-picked Sacramento fruit was low during the initial run, but within 24 hours the rate rose almost to the maximum.

Again there is a rough parallel between the probable total number of milligrams of CO_2 given off and the degree of ripeness of the fruit, regardless of temperature. In the early-picked Sacramento fruit held at 60° F. approximately 10,800 mgm. of CO_2 per kilogram of fruit were given off before the fruit was respiring at a rate of 50 mgm. per kilogram hour, the initial rate when the fruit was removed from cold storage. At a rate of 3 mgm. per kilogram hour, this would be equivalent to about five months in cold storage. This fruit had actually been in storage about seven months when these tests were begun. Calculating the early-picked Santa Clara and the late-picked Sacramento fruit in the same way, it is found that in each case the fruit coming out of cold storage had an initial respiration rate somewhat below that of similar fruit held after picking at 60° F. until it had given off an equal total quantity of CO_2.

It is thus apparent that after coming out of cold storage, Bartlett pears do not respire faster than fruit of the same degree of ripeness that has not been in cold storage. It is impossible, however, to entirely stop the ripening processes by cold storage, unless the fruit is actually frozen. The reduction in the time that the fruit will hold up following removal from storage depends on the total amount of ripening that has occurred during cold storage, which varies with the length of time and the temperature in storage.

RESPIRATION OF PEARS IN NITROGEN

At various times during these present investigations, Bartlett pears have been entirely sealed from the air by immersing them for an instant in melted paraffin. Invariably such treatment almost entirely checked the ripening processes. With this fact in mind, in order to test the evolution of CO_2 from the fruit in the absence of O_2 and to throw some light on the processes involved in pear ripening, the CO_2 output in an atmosphere of N_2 was measured.

Oxygen was absorbed from air by ammonia, ammonium carbonate, and copper, using an apparatus similar to that described by Van Brunt (*18*). After passing through the oxygen-absorption apparatus, the gas was bubbled through sulphuric acid to remove any ammonia. Then it passed through the soda-lime towers, and through the fruit chambers to the Reiset absorption tubes. To operate the air lift of the oxygen-absorption apparatus it was necessary to operate the respiration chamber under a partial vacuum, amounting to about 4 inches of mercury. Consequently, the check experiment operated with air and using fruit of the same pick and which had been held under the same conditions up to the time of starting the run, was tested under a similar reduced pressure. The fruit used in these runs was late-picked Sacramento pears, held at 30° F. until September 1, or 18 days after picking. The data on these runs are given in tables XIV and XV and in curves 3, 3A, and 2 of Figure 2.

TABLE XIV.—*Respiration of late-picked Bartlett pears from the Sacramento Valley, Calif., in an atmosphere of nitrogen, and in air following nitrogen*

Run No.	When started	Length of run	Kilogram hours	Weight of CO_2 given off	CO_2 per kilogram hour	Average temperature
		Hours		*Gm.*	*Mgm.*	° *F.*
1	Sept. 1, 5.30 p. m.	22.08	81.9	0.831	10.15	60.0
2	Sept. 2, 3.35 p. m.	24.67	91.5	1.627	17.78	60.0
3	Sept. 3, 4.15 p. m.	23.33	86.5	0.941	10.87	60.0
4	Sept. 4, 3.35 p. m.	42.75	158.6	1.191	7.51	59.5
5	Sept. 6, 10.20 a. m.	52.92	196.3	1.183	6.03	58.5
6	Sept. 8, 3.15 p. m.	49.67	184.2	0.772	4.19	57.5
7	Sept. 10, 4.55 p. m.	66.5	246.6	0.928	3.76	56.0
8	Sept. 13, 11.25 a. m.	51.5	191.0	0.828	4.33	55.0
	AIR FOLLOWING NITROGEN					
1A	Sept. 15, 3.00 p. m.	44.0	163.2	1.588	9.73	55.0
2A	Sept. 17, 11.00 a. m.	45.58	169.2	2.340	13.84	55.0
3A	Sept. 19, 8.35 a. m.	48.83	181.1	3.381	18.67	55.0
4A	Sept. 21, 9.25 a. m.	53.08	196.9	5.091	25.86	55.5
5A	Sept. 23, 2.30 p. m.	50.25	186.4	6.010	32.25	55.5

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TABLE XV.—*Check on respiration in nitrogen of late-picked Bartlett pears from the Sacramento Valley, Calif., in air under reduced pressure equal to 4 inches of mercury*

Run No.	When started	Length of run	Kilogram hours	Weight of CO_2 given off	CO_2 per kilogram hour	Average temperature
		Hours		*Gm.*	*Mgm.*	° *F.*
1	Sept. 1, 6.00 p. m.	22.0	83.65	1.488	17.79	60.0
2	Sept. 2, 4.00 p. m.	47.75	181.6	4.085	22.50	60.0
3	Sept. 4, 3.45 p. m.	43.58	165.7	4.326	26.11	59.5
4	Sept. 6, 11.20 a. m.	52.17	198.4	6.986	35.21	58.5
5[a]						
6[a]						
7	Sept. 11, 4.30 p. m.	42.58	161.9	5.104	31.52	56.0
8	Sept. 13, 11.05 a. m.	51.5	195.8	6.817	34.81	55.0

[a] Runs lost.

From the data presented in Table XIV it is evident that considerable carbon dioxide was given off in the absence of oxygen, especially during the first three days. After that there was a steady decrease, which continued until the air was admitted to the chamber. During the last run in nitrogen, there was a slight increase in CO_2 given off, but it was thought that a small amount of oxygen passed into the desiccator containing the fruit, due to exhaustion of the reagents in the O_2 absorption apparatus.

Following the admission of air to the fruit chamber, respiration rapidly increased, but not to the full extent of the check run in air. The fruit ripened and was normal in appearance, but was not edible. Certain products formed by the intramolecular respiration, which must have occurred to produce CO_2 in the absence of O_2, rendered the fruit disagreeable to the taste. These products, notably alcohol, may also have interfered with normal respiration following the admission of oxygen.

It is also apparent that the check operated under a reduced pressure equivalent to 4 inches of mercury (Table XV) did not respire as rapidly when soft-ripe as did lots of the same fruit just picked from the tree (Table III) or when held in storage much longer and then removed and tested under normal pressure (Table XII). The maximum rate attained by this fruit was about 35 mgm. per kilogram hour, whereas both earlier and later runs attained a rate of 48 to 49 mgm. Although the temperature was slightly lower during this run, it was hardly sufficient to account for the marked difference. It seems probable that the difference in air pressure under which the tests were conducted affected the rate of CO_2 evolution.

CATALASE CONTENT AND OXYGEN SUPPLY IN RELATION TO RESPIRATION

From a consideration of all of the above data, it is apparent that temperature is not the only limiting factor in Bartlett-pear respiration. Absence of oxygen almost inhibited carbon-dioxide output, and the rate of carbon-dioxide evolution was also dependent to a very large extent upon the degree of ripeness of the fruit.

The enzyme catalase has often been suggested as being intimately associated with respiratory activity of plant and animal tissue. Many workers have reported a close association between the catalase

activity of tissue and its respiratory activity. Recently Mrs. Rhine (*17*) and Magness and Burroughs (*14*) have reported that increased catalase activity seems to follow respiratory activity rather than being the cause of it. Heinicke (*8, 9*) has suggested the value of the catalase reaction as a measure of total metabolic activity in tissue. During the season following the one in which the respiration of the fruit had been determined, a series of studies was made to see whether or not the marked variation in respiratory activity due to stage of maturity was associated with similar changes in catalase content.

Another subject that seemed deserving of attention was the composition of the intercellular atmosphere of pears at various stages of ripening. One of the writers (*12*) has shown that there is a relative increase in the carbon dioxide and a decrease in the oxygen content within the tissue of apples and potatoes when the temperature at which they are held is raised. Magness and Diehl (*15*) have shown that an accumulation of carbon dioxide in apple tissue tends to inhibit the respiratory activity of that tissue. It therefore seemed of interest to determine whether the change in respiration rate during the ripening of pears was a result of increased oxygen supply in the tissues. Previous work had shown the fruit to give off carbon dioxide only very slowly in the absence of oxygen.

Obviously it would have been desirable to conduct these experiments on catalase and intercellular atmosphere parallel with the respiration experiments, so that the same general lot of fruit could have been used. Lack of time, however, made it necessary to postpone the work on catalase and intercellular gas until the following season.

On August 4, 1921, two boxes of pears were shipped from the same Sacramento orchard from which the fruit for the respiration studies of 1920 had been obtained. These boxes were selected from the regular commercial pack, picked in a condition of maturity intermediate between the two pickings of the previous season. The fruit arrived in Watsonville, Calif., and was placed in storage at 28° F., on August 6, 1921.

APPARATUS AND METHODS FOR CATALASE DETERMINATIONS

The apparatus used for catalase determinations was similar in principle to that described by Appleman (*1*). It consisted of an 8-ounce glass cylinder with a rubber stopper and glass agitator which revolved about 1,000 times per minute. The steel shaft to which the agitator was cemented turned in a gas-tight, mercury-sealed bearing (*16*) which passed through the center of the rubber stopper. A 30-milliliter separatory funnel, provided with a stopcock, held the hydrogen peroxide, as in Appleman's apparatus. The water bath in which the reaction bottle was immersed was held at a temperature of 25° C. $\pm 0.1°$ C., by means of a thermostat. Two 100-milliliter burettes were connected to a manifold which in turn was connected to the reaction bottle by means of rubber tubing. A single 100-milliliter burette was not sufficient to hold all of the gas liberated in some of the determinations, and having two burettes available facilitated collecting the gas and avoided the possibility of losing some of the sample. All of the gas burette readings were corrected for temperature, but barometric corrections were not made.

The sample of tissue for catalase determination was a flat, triangular-shaped piece of tissue cut from the side of the pear, in such a way as to proportionally represent the different regions of the fruit. The skin was removed from this section of tissue, which was trimmed down to 2 grams. No account was taken of variation in water content of different pears. The 2-gram sample was at once ground to a smooth paste, with approximately 1 gram of very fine calcium carbonate. The thoroughly ground sample was washed from the mortar into the reaction bottle with 15 c. c. of distilled water, 20 c. c. of hydrogen peroxide was put into the separatory funnel, and the reaction bottle was clamped in the water bath to bring its temperature to that of the bath. As soon as the cylinder was clamped in the bath the agitator was started. A method was provided whereby any change in the volume of the air in the apparatus caused a short column of water to move along a capillary tube, and by means of this device it was possible to determine very accurately when the air and the pear tissue in the reaction cylinder had reached the temperature of the bath. When the bath temperature was reached, the expanded air was allowed to escape through stopcocks, and the determination was started by allowing the H_2O_2 to run into the reaction cylinder. By gradually lowering the bulb connected with the gas burette the gas was kept under practically atmospheric pressure, and readings of the gas volume could be made at regular intervals. During the first few minutes, with tissue high in catalase content, the evolution of oxygen was so rapid as to make very accurate readings difficult. In the determinations Series I to Series VI, inclusive, the gas volume was read at intervals of one minute, the cock at the bottom of the gas burette being closed for a few seconds while the reading was being made, and 15 of these 1-minute readings were made for each pear sampled. In Series VII to XV, inclusive, only one reading was made, this at the end of the 15-minute interval.

It was found in preliminary experiments that the rate of evolution of gas at the end of a 15-minute period was so low that a period of this length might be taken as the standard, for the purposes of the present work. The writers realize that this method of measuring catalase activity by the volume of gas evolved from hydrogen peroxide during a fixed period is open to objections, but data resulting from it are comparable and serve to show the differences which exist.

A brand of hydrogen peroxide containing no preservative was used. The hydrogen-peroxide concentration of different bottles varied from 0.6 per cent to more than 3 per cent, the latter being the guaranteed original strength. The oldest product, however, as indicated on the label, was not always the weakest. A composite sample containing 3 per cent of hydrogen peroxide was prepared, sufficient in quantity for all the experiments on one box of pears. This stock sample was carefully neutralized to phenolphthalein before use. The strength of the peroxide solution was determined by titration with potassium permanganate, and as 1 volume of 3 per cent hydrogen peroxide is capable of yielding approximately 10 volumes of oxygen, the 20 c. c. used in each determination was capable of yielding about 200 c. c. of oxygen, which is a larger volume of gas than was recorded in any of the experiments.

The apparatus used for extracting the gas from pear tissue was essentially the same as that already described by one of the writers (*12*). Samples of tissue were cut with a steel cork borer. The borer was forced diametrically crosswise through the pear where it was widest, the sample thus including part of the core region. The gas samples were analyzed for carbon dioxide and oxygen by the Bonnier-Mangin method (*2*). The Bonnier-Mangin apparatus used was improvised in the laboratory, but the capillary measuring tube was carefully calibrated by means of a short mercury column, and calibration corrections were applied to all the readings, each recorded reading being the average of two successive readings, or three in case of appreciable discrepancy.

CATALASE EXPERIMENTS

The two boxes of pears placed in cold storage at 28° F., August 6, 1921, will be designated as box A and box B. The following schedule summarizes the history of the fruit from box A that was used in catalase determinations.

SCHEDULE I.—BOX A. CATALASE

1921

August 4. Fruit shipped from Sacramento, Calif.
6. Fruit placed in storage at 28° F. at Watsonville, Calif.
12. All but about 25 pears transferred to ripening room at temperature of 59°.
Series I, pears 1 to 6, inclusive, taken directly from 28° room to laboratory.
13. Series II, pears 7 to 12, inclusive, 24 hours in 59°, but fruit temperature was still somewhat below 59°.
17. Series III, pears 13 to 22, inclusive, 5 days in 59°.
20. Series IV, pears 23 to 33, inclusive, 8 days in 59°.
24. Series V, pears 34 to 42, inclusive, 12 days at 59°. Slightly past prime eating condition, flesh beginning to turn brown at core.
29. Series VI, pears 43 to 52, inclusive, 28° storage continuously since Aug. 6.

From August 6 (when the fruit was first put in storage) until August 16, the temperature of the so-called 28° F. room ran fairly constant at the specified temperature, the maximum variation being not more than 1 degree. During a period of 2½ days from August 16, however, the temperature rose to 36°, and then dropped back to 28°. The temperature of the ripening room ran constantly between 59° and 60° during the ripening period, as shown by the thermograph records.

The results of the experiments on pears in box A are given in Tables XVI and XVII. The gas production during 15 minutes is given for each of the 6 to 10 pears used on each series in Table XVI, and the previous history of the fruit used in each series can be obtained by reference to Schedule I.

TABLE XVI.—*Catalase determination of Bartlett pears from box A. Total oxygen (in cubic centimeters) liberated by 2 grams of tissue from each pear, during run of 15 minutes, at 25° C.*

Series I		Series II		Series III		Series IV		Series V		Series VI	
Pear No.	Cubic centimeters	Pear No.	Cubic centimeters	Pear No.	Cubic centimeters	Pear No.	Cubic centimeters	Pear No.	Cubic centimeters	Pear No.	Cubic centimeters
1	77.5	7	95.0	13	117.1	23	150.9	34	89.3	43	126.0
2	88.6	8	85.6	14	154.6	24	165.2	35	61.9	44	111.9
3	100.2	9	111.5	15	128.4	25	126.9	36	82.4	45	117.1
4	92.4	10	135.7	16	149.1	26	120.2	37	99.9	46	123.0
5	109.6	11	143.1	17	148.2	27	163.2	38	76.2	47	125.4
6	106.0	12	102.2	18	131.3	28	108.5	39	88.2	48	113.3
				19	162.6	29	146.4	40	55.1	49	144.4
				20	139.5	30	96.6	41	86.4	50	154.8
				21	163.8	31	118.4			51	148.7
				22	147.1	32	105.8			52	133.9
Average	95.7	Average	112.1	Average	144.4	Average	130.1	Average	80.1	Average	129.9

TABLE XVII.—*Catalase determination. Average amount of oxygen (in cubic centimeters) liberated each minute by pears of Series I to VI*

Minute	Series I	Series II	Series III	Series IV	Series V	Series VI
	Pears 1 to 6, inclusive	Pears 7 to 12, inclusive	Pears 13 to 22, inclusive	Pears 23 to 32, inclusive	Pears 33 to 42, inclusive	Pears 43 to 52, inclusive
First	32.5	36.0	50.4	48.0	31.0	43.3
Second	17.9	21.8	26.9	24.7	16.0	24.8
Third	11.4	14.4	17.7	15.8	10.1	16.9
Fourth	7.8	10.3	12.5	10.7	6.7	11.8
Fifth	5.7	7.4	8.9	7.7	4.7	8.5
Sixth	4.4	5.7	6.6	5.8	3.4	6.4
Seventh	3.3	4.3	5.1	4.4	2.3	4.8
Eighth	2.9	3.3	4.0	3.3	1.7	3.7
Ninth	2.2	2.5	3.2	2.6	1.2	2.7
Tenth	1.9	1.9	2.4	2.0	0.9	2.1
Eleventh	1.6	1.5	2.0	1.5	0.7	1.6
Twelfth	1.3	1.1	1.6	1.2	0.5	1.2
Thirteenth	1.1	0.9	1.3	1.0	0.4	0.9
Fourteenth	1.0	0.7	1.0	0.8	0.3	0.7
Fifteenth	0.9	0.6	0.8	0.6	0.2	0.5
Total	95.9	112.4	144.4	130.1	80.1	129.9

The detailed results for each pear given in Table XVI show the variation among the individual pears of a series, while the data in Table XVII give an idea of the general course of reaction. The curve for box A in Figure 4 is a graphic presentation of the mathematically averaged data given in Tables XVI and XVII. The dotted line connecting Series I and VI indicates the change in catalase content in fruit of box A while in storage at 28° F. or slightly above, while the solid line connecting Series II to V represents changes in catalase content in pears held at 59°.

During the first 24 hours after the fruit was transferred from the 28° F. storage room to the 59° ripening room the catalase content increased rapidly, the liberation of oxygen increasing from 95.9 c. c. to 112.1 c. c. during this time. The average of Series III, from fruit which had been in the ripening room five days, was 144.4 milliliters of oxygen, indicating a still further rapid rise in catalase content and representing the highest point attained by any of the fruit. The significance of the various average totals shown in Table XVII

is more plainly brought out in Figure 4, curve 1. On the line joining Series IV and V, a point corresponding to the date of the full yellow-ripe or prime eating condition of the fruit has been indicated. From this it will be seen that the catalase content of the fruit had probably reached its maximum about five days previous to the development of the best eating quality, and that at the time of prime eating condition the catalase content was decreasing rapidly, so that at the time of the last determinations (Series V on August 24) the catalase content was less than at any other time during the experiments. Series VI represents fruit that remained in the 28° room until August 29, or 17 days after the fruit in Series I was taken out. The average catalase content of the fruit was markedly above that of Series I, indicating that even at the low temperature storage used here the catalase content increased during the time in storage.

On September 14 another set of experiments, Series VII to XV, was started, using the fruit of box B. The following schedule summarizes the history of the fruit used in each series and includes notes on the temperature of the storage and ripening rooms.

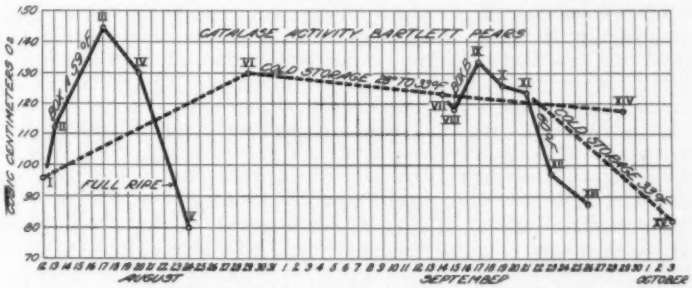

FIG. 4.—Catalase activity in Bartlett pears, held in cold storage, and at 60° F.

SCHEDULE II.—BOX B. CATALASE

1921

August 4. Fruit shipped from Sacramento, Calif.

6. Fruit placed in storage at 28° F. at Watsonville, Calif.

16. The temperature of the storage room rose to 36° for 2½ days, then dropped back to 28°.

September 9. The temperature of the storage room rose to 36°, dropped back to 33°, and remained between 33° and 34° until the conclusion of the experiments with fruit from box B.

14. All but 20 pears of box B were taken from the 33° storage room to the ripening room. A very slight tinge of yellow was showing on some.

Series VIII, pears 53 to 62, inclusive, taken immediately to the laboratory from the 33° storage room for catalase and internal gas analyses. Fruit green and hard, except for a few pears showing a slight yellowish tinge, as mentioned above.

15. Series VIII, pears 63 to 70, inclusive, taken from the ripening room at end of 24 hours.

17. Series IX, pears 73 to 80, inclusive, after three days in ripening room. Softening and turning yellow.

19. Series X, pears 81 to 89, inclusive, five days in ripening room. Almost full yellow, but moderately firm.

21. Series XI, pears 90 to 100, inclusive, except 94, 95, and 98, seven days in ripening room. Full yellow, but too firm for prime eating condition.

23. Series XII, pears 101 to 108, inclusive. Nine days in ripening room. Prime eating condition. Full ripe and yellow.

1921

September 26. Series XIII, pears 109 to 116, inclusive. Twelve days in ripening room. Past prime eating condition. Flesh discolored and breaking down at core. Flavor gone.

29. Series XIV, pears 117 to 124, inclusive. Fruit held continuously in 28° to 33° F. storage from August 6 until removed for testing.

October 3. This fruit was transferred from the cold storage to the ripening room on September 14. After seven days in the ripening room, at temperatures averaging 57° to 62°, the pears were returned to the cold-storage room at 33°, where they were left until October 3. When returned to cold storage this fruit had not yet reached prime eating condition. On October 3 much scald had developed, but the taste was fairly good.

The results of the tests on the fruit in box B are shown in Table XVIII. Under each series is given the total gas production in cubic centimeters at the end of 15 minutes for each pear, and the mathematical average for the series. These data are also presented graphically in Figure 4, curve for box B. When removed from the cold room this lot of pears had a high catalase content, as shown by the fact that Series VII averaged 123.1 c. c. of oxygen liberated. This fruit had been in cold storage 39 days, and apparently had increased considerably in catalase content during that time. In general, fruit from box B seemed to be near its maximum catalase content when removed from cold storage. It was slightly higher after three days in the ripening room (Series IX) than when removed from cold storage, then dropping off rather sharply with longer holding in the ripening room. The broken line in Figure 4, connecting Series I, VI, VII, and XIV, represents the catalase activity in fruit held continuously in cold storage at temperatures of 28° to 33° F. It is apparent that during the first 17 days in cold storage the catalase content increased markedly, after which it remained almost stationary with a slight dropping off until the end of the test.

Fruit removed to the ripening room at temperatures approximating 60° F. showed a sharp increase in catalase activity, as compared with the activity at the beginning of the cold-storage period. Fruit held for longer than a month in cold storage did not show as marked an increase in activity when removed to 60° temperature, partly because the cold-storage rate was higher at that time.

TABLE XVIII.—*Catalase determination. Data from each pear in each series from box B, representing total cubic milliliters of gas liberated during 15 minutes, the entire period of each determination*

Series VII, Sept. 14		Series VIII, Sept. 15		Series IX, Sept. 17		Series X, Sept. 19		Series XI, Sept. 21	
Pear No.	Milliliter of O_2	Pear No.	Milliliter of O_2	Pear No.	Milliliter of O_2	Pear No.	Milliliter of O_2	Pear No.	Milliliter of O_2
53	97.6	63	125.4	73	159.0	81	131.0	90	163.3
54	126.8	64	126.1	74	139.0	82	148.6	91	114.5
55	109.0	65	106.3	75	121.2	83	157.4	92	118.8
56	149.4	66	115.6	76	145.1	84	109.6	93	115.4
57	109.8	67	118.1	77	134.3	85	118.2	96	131.4
58	141.5	68	97.2	78	113.4	86	93.7	97	102.9
59	144.9	69	143.3	79	130.8	87	144.4	99	135.2
60	119.9	70	109.8	80	126.6	88	126.2	100	106.6
61	130.2					89	104.4		
62	101.9								
Average	123.1	Average	117.7	Average	133.7	Average	125.9	Average	123.5

TABLE XVIII.—*Catalase determination. Data from each pear in each series from box B, representing total cubic milliliters of gas liberated during 15 minutes, the entire period of each determination*—Continued

Series XII, Sept. 23		Series XIII, Sept. 26		Series XIV, Sept. 29		Series XV, Oct. 3	
Pear No.	Milliliter of O_2	Pear No.	Milliliter of O_2	Pear No.	Milliliter of O_2	Pear No.	Milliliter of O_2
101	70.8	109	87.7	117	127.8	125	50.2
102	93.2	110	94.1	118	127.5	126	101.4
103	95.7	111	116.1	119	113.5	127	82.3
104	100.5	112	103.5	120	135.7	128	72.2
105	113.4	113	58.4	121	87.7	129	75.2
106	115.2	114	102.5	122	125.6	130	100.8
107	86.8	115	66.2	123	101.7	131	93.7
108	99.4	116	70.7	124	121.6		
Average	96.9	Average	87.4	Average	117.6	Average	82.3

Obviously, there is not a particularly close association between catalase activity and respiration in Bartlett pears. The respiration rate at cold-storage temperatures remained practically constant, whereas the catalase activity increased during the first two or three weeks. At 60° F. the respiration rate increased greatly as the fruit ripened, not reaching a maximum rate of CO_2 evolution until the fruit was full soft and in prime eating condition, and not beginning to decrease until the fruit was breaking down. Catalase activity also increased for a time in pears held at 60°, especially in fruit previously held for only a short time at cold-storage temperatures. The maximum catalase activity in all cases, however, was in pears not quite to the full-ripe condition after which the activity decreased markedly. At the time the pears were almost full ripe, the respiratory activity was increasing while catalase was decreasing rather rapidly. Thus the highest point in catalase activity came sometime prior to the period of greatest respiratory activity.

At the time of making the determinations for series XI (fig. 4) one lot was transferred from the ripening room back to 33° F. and held for 12 days. Catalase activity in this lot (series XV) was greatly reduced from the activity when the lot was removed from the ripening room. Activity was decreasing when this fruit was removed from the ripening room, and it apparently continued to decrease under cold-storage temperature.

Magness and Burroughs (*14*) have determined the catalase activity of apple tissue, using exactly the same quantities of tissue as were used in the present investigation. After two weeks at a temperature of 65° F., Baldwin apples gave up about 25 c. c. of oxygen from a 2-gram sample, and Winesap yielded less than 15 c. c. Bartlett-pear tissue yielded as much as 140 c. c. of oxygen under similar conditions. Apparently Bartlett pears contain six to eight times as much catalase as these varieties of apples. Heinicke (*8, 9*) has emphasized the value of catalase determinations as a measure of the total metabolic activity of plant tissue. Bartlett pears are highly perishable as compared with Baldwin and Winesap apples, respiring faster, and softening far more rapidly. In this instance, high catalase content seems to be associated with high metabolic activity, as Heinicke found in apple leaf and twig studies. How-

ever, it does not seem that there is a causal or even intimate relationship between respiration and catalase activity in Bartlett pears.

It should be pointed out that a marked variation occurred in the determinations of catalase activity in the different pears of a series, and in certain cases even in duplicate tests from a single pear when every effort was made to standardize the technique. A larger number of individual determinations would have given more accurate averages. However, the averaged data, as summarized in Figure 4, are sufficiently consistent to give a very clear indication of the catalase changes which occur in ripening Bartlett pears.

COMPOSITION OF THE INTERCELLULAR ATMOSPHERE OF PEARS

The fruit used in determining the composition of the gas in the intercellular spaces of Bartlett pears was from the same boxes A and B used in the catalase work. The fruit of box A arrived at Watsonville August 6, was held six days in cold storage, and then most of it was placed in the ripening room. Series I represents fruit in cold storage at 28° F.; Series II to VI, fruit exposed for varying lengths of time to ripening-room temperatures of 59°; Series VII, fruit held continuously in cold storage to August 24; Series VIII was

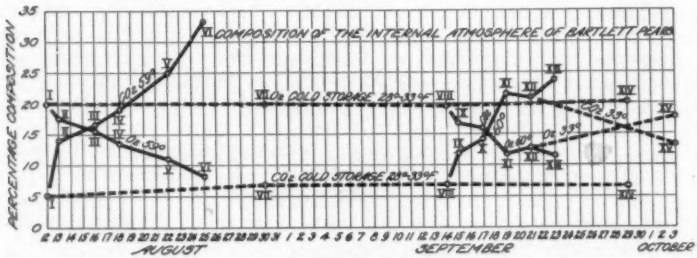

FIG. 5.—Internal oxygen and carbon-dioxide contents of Bartlett pears, held in cold storage and while ripening at 60° F.

fruit from box B held in cold storage until September 14 (33 days); Series IX to XIII, inclusive, were removed from the cold-storage room on September 14 and held for varying lengths of time in the ripening room at a temperature of about 60°; Series XIV again represents fruit held continuously in cold storage; and Series XV was fruit taken from the ripening room September 21, when almost full-ripe, and held until October 3 at about 33°. The condition of ripeness of this fruit can be determined from Schedules I and II; the dates of testing are apparent from Table XIX and from the curves in Figure 5. The gas was extracted from the intercellular spaces and core region of the individual pears, and the percentages of oxygen and carbon dioxide were determined as outlined above.

The detailed data for each pear and the mathematical averages of the seven series of determinations from box A are shown in Table XIX. The averages of the various series are also graphically shown in Figure 5.

TABLE XIX.—*Carbon-dioxide and oxygen content of intercellular gas in Bartlett pears from the Sacramento Valley, Calif., August, 1921*

[Data from the individual pears from each of the seven runs]

Series, date, and pear No.	Per cent CO_2	Per cent O_2
Series I, Aug. 13		
1	2.7	20.5
2	5.8	20.5
3	4.7	20.8
4	5.5	19.7
5	3.3	19.9
6	8.3	17.7
Average	5.1	19.9
Series II, Aug. 13		
7	13.4	17.0
8	13.9	17.6
9	13.9	17.8
10	16.0	16.2
11	11.0	17.7
12	14.1	17.6
Average	13.7	17.3
Series III, Aug. 16		
13	12.6	16.5
14	15.9	15.7
15	18.1	14.5
16	16.6	15.2
17	15.0	15.7
18	13.2	15.5
19	16.2	15.4
20	19.8	14.4
21	19.7	14.5
22	15.7	15.5
Average	16.3	15.3
Series IV, Aug. 18		
23	20.3	12.6
24	23.9	11.5
25	18.8	13.0
26	13.1	14.7
27	26.2	10.6
28	13.1	16.0
29	20.1	13.6
30	20.4	12.0
31	14.2	15.6
Average	18.9	13.3
Series V, Aug. 22		
32	29.5	9.0
33	21.5	12.2
34	29.8	8.8
35	13.3	15.9
37	21.7	10.9
38	28.7	10.2
39	23.6	11.9
40	31.2	8.0
41	25.1	9.5
Average	24.9	10.7
Series VI, Aug. 25		
42	28.0	9.5
43	26.8	10.7
44	24.2	11.1
45	26.2	10.1
46	32.0	7.8
47	43.3	5.5
Series VI, Aug. 25.— Continued		
48	41.9	7.3
49	41.9	5.9
50	35.4	6.2
Average	33.3	8.2
Series VII, Aug. 30		
51	4.0	20.6
52	13.2	17.4
53	5.4	21.7
54	6.9	21.5
55	6.2	21.3
56	3.9	22.1
57	4.7	20.3
58	5.7	21.3
59	8.0	15.7
60	10.8	16.9
Average	6.9	19.9
Series VIII, Sept. 14		
61	4.0	20.8
62	3.5	21.1
63	5.7	20.4
64	5.2	20.6
65	6.4	19.5
66	9.7	18.2
67	9.2	18.8
68	9.7	18.2
69	7.6	19.2
70	8.0	19.2
Average	6.9	19.6
Series IX, Sept. 15		
71	14.2	16.2
72	9.7	16.9
73	11.4	15.9
74	10.8	17.1
75	10.9	17.7
76	16.3	15.6
77	7.3	18.0
78	13.7	16.2
Average	11.8	16.7
Series X, Sept. 17		
79	12.9	16.3
80	15.1	16.1
81	14.6	16.2
82	12.4	15.8
83	14.8	15.8
84	17.8	14.1
85	13.8	15.3
86	11.1	16.6
Average	14.1	15.8
Series XI, Sept. 18		
87	17.0	14.3
88	13.2	15.0
89	25.9	9.4
90	19.8	12.2
91	24.2	11.4
92	14.6	14.6
93	28.2	8.8
94	28.7	8.9
Average	21.5	11.8
Series XII, Sept. 21		
95	18.6	13.7
96	14.6	15.8
97	21.0	12.8
98	17.0	14.1
99	18.4	14.0
100	24.4	10.5
101	21.8	12.6
102	31.7	7.8
Average	20.9	12.7
Series XIII, Sept. 23		
103	32.1	7.2
104	23.4	10.5
105	20.9	13.2
106	29.0	9.6
108	22.3	12.6
109	10.7	17.0
110	28.1	9.7
Average	23.8	11.4
Series XIV, Sept. 29		
111	10.9	19.1
112	7.7	20.1
113	4.3	21.3
114	5.6	20.3
115	5.5	19.6
116	8.9	19.9
117	6.4	20.0
118	4.6	20.5
Average	6.7	20.1
Series XV, Oct. 3		
119	12.2	18.4
120	10.8	18.5
121	8.8	19.3
122	10.9	19.1
123	17.6	14.4
124	15.5	16.6
125	16.2	16.6
Average	13.1	17.6

It is natural to expect that, due to respiration, the CO_2 content of the intercellular atmosphere would be higher than that of normal air, even at 28° F., since normal air contains approximately only 0.03 per cent of CO_2 (*3*). Series I, VII, VIII, and XIV show the composition of the internal atmosphere of pears held continuously in storage at 28 to 33°. The CO_2 content averaged from 5.1 to 6.9 per cent in these series, while the average oxygen content varied from 19.6 to 20.1 per cent, which is only slightly below Benedict's figure (20.94 per cent) for normal air. On October 13, five successive analyses of out-of-doors town air, using the same technique of analysis as for the gas from pears, gave 20.78, 20.23, 20.64, 20.36, and 20.45 per cent oxygen. All of these figures are below Benedict's final calculation, as is also their average (20.49 per cent). The above figures also indicate something of the variation that may be expected in successive analyses from the same sample of gas when a technique in keeping with a reasonable speed of routine work is employed.

It is apparent that in pears held continuously at cold-storage temperatures there is little variation in either the carbon-dioxide content or the oxygen content. Since the respiration rate changes little under these conditions, it would seem that there was little change in the permeability of the skin and other tissue during the time the fruit was held in cold storage.

When the fruit was removed to the ripening room, and held at temperatures of 59 and 60° F., there was at once a fairly sharp increase in the carbon-dioxide content of the tissue, and a slight decrease in oxygen. This is in accord with the findings of one of the writers (*12*) for apples and potatoes—that the higher the temperature at which the products were held, the higher the carbon dioxide and the lower the oxygen content in the tissues.

Prior to making these tests it was thought that as pears ripen the permeability of the tissue might increase, and the increased respiration rate be thus due, in part at least, to the better aeration of the tissues. The results of the tests recorded under Series II to VI, inclusive, show, however, that as Bartlett pears ripen at temperatures around 60° F. there is a marked increase in the concentration of carbon dioxide in the tissues, and a considerable decrease in oxygen. So it is apparent that aeration of the tissues is less complete in fruit in prime eating condition than in hard green pears. The permeability of the tissue in pears held at 60° F. does not increase nearly as rapidly as does the rate of liberation of CO_2 within the tissues.

It is interesting to note, however, that even in ripe pears held at 60° F. there was a considerable concentration of oxygen in the tissues. In only one series was the oxygen in the extracted gas below 10 per cent, though the carbon dioxide in the ripe pears ranged from 20 to more than 30 per cent. This strongly suggests that the respiration of Bartlett pears may be in part intramolecular, with much more CO_2 being formed than oxygen taken up. On the other hand, it may be due to a more rapid diffusion of oxygen inward than of CO_2 outward, though this seems improbable.

A study of the data for individual pears in Table XIX shows wide variations in the composition of the gas from the intercellular space of different pears, just as in the determinations for catalase activity.

The averages indicate clearly, however, the marked increase in CO_2 and decrease in oxygen in the tissues as the pears ripen at higher temperatures.

On September 21 one lot of fruit (Series XV) was removed from the ripening rooms to cold storage. After 12 days this lot showed a much lower carbon-dioxide content and a higher oxygen content than when removed from the higher temperature, but had not yet reached the level of pears continuously in cold storage. This fruit was somewhat scalded at the time of final testing, which may have affected its respiratory activity.

It is apparent from the data presented that the internal atmosphere of Bartlett pears varies with the respiration activity. When the respiration rate is low, there is a relatively low concentration of carbon dioxide and a high concentration of ogygen in the tissues; at higher temperatures, with the increase in respiration rate, the carbon-dioxide content increases and the oxygen decreases. As the fruit ripens, increased respiration rate is accompanied by similar changes in the composition of the intercellular atmosphere.

DISCUSSION

The results obtained in measuring the amounts of carbon dioxide given off by Bartlett pears have been distinctly different from those recorded for fleshy fruits by previous investigators. They clearly indicate the possibility of erroneous conclusions from short and isolated measurements of CO_2 evolution by fleshy vegetable organisms.

There is a very marked increase in CO_2 evolution by Bartlett pears from the time of picking until they are fully ripe. This increase is more marked than would be the variation caused by wide ranges of temperature. Gore (*7*), in his extensive studies on fruit respiration, failed to note any similar increase in the fruits studied, but usually only one run was made at a given temperature on any lot of fruit. Gerber (*6*), working with immature apples and other highly-acid fleshy fruits, reports that at temperatures of 30° to 33° C. (86° to 91° F.) the CO_2 output of these fruits rapidly diminishes. The acid also disappears rapidly, and at these high temperatures the amount of CO_2 given off by the fruit is markedly greater in volume than the O_2 absorbed. At 18° C. (64° F.), however, both the CO_2 output and the acidity of the fruit remained fairly constant. At temperatures below 18°, particularly at 0° C. (32° F.), he found the CO_2 given off to be less in quantity than the O_2 absorbed. In general, he found the same condition to hold in grapes and other acid fruits studied. In no case did he find a marked and consistent increase in CO_2 evolution in acid fruits. However, in certain fruits with a high tannin content he did report a more or less continuous and marked increase in the quantity of CO_2 evolved.

Hill (*10*), in a rather extensive series of studies on the effect of storing a number of fruits in air, nitrogen, and hydrogen, found a slight increase in the respiration of cherries at 30° C. in air during progressive experiments. He suggests the possible production of an autocatalytic enzyme during the ripening of the fruit. There was a decrease in respiratory rate with green peaches, however, thus corresponding to the findings of Gerber. With grapes there seemed little variation from the first until the last runs.

Since the studies reported here were made, Magness and Burroughs (*14*) have reported that there is a slight but apparent drop in respiration rate in Winesap and Baldwin apples while being held at 65° F. Burroughs (*5*) found that Wagener and Wealthy apples showed a distinct increase in respiration rate when held at 68.5° F. from the time they were picked until soft-ripe. This increase was similar to that reported here for Bartlett pears, but was much less marked.

While there was no increase in the respiration rate of fruit held at temperatures approximating 30° F., fruit held for a period at this temperature, then placed in 60°, started off at a rate much faster than similarly picked fruit that had not been in cold storage but which was tested for CO_2 output at 60° immediately after picking. This would seem to indicate that if an autocatalytic enzyme is produced, as Hill (*10*) suggests, it must develop in comparatively large quantities at cold-storage temperatures, but at the same time it must remain inactive at low temperatures.

Catalase, which in many plant tissues seems to be associated with respiration and general metabolic activity, increases for a time as the fruit ripens, but then decreases much sooner than does the respiration rate. It is apparent that the marked increase in respiration as the fruit ripens can not be associated with the production of catalase as a causal agency.

It was thought during the first season's work that the supply of oxygen within the tissues might be the principal limiting factor in CO_2 output from Bartlett pears when held at about 60° F., but apparently this is not true, for there was a greater quantity of oxygen in the tissues of the green fruit than in similar fruit when ripe and soft. Also, the CO_2 concentration within the tissues was greater in the ripened fruit during the period of greatest CO_2 evolution. Consequently, lack of O_2 or accumulation of CO_2 within the tissues will not account for the slower respiration rate in unripe fruit.

SUMMARY

This report deals with measurements of the CO_2 given off by Bartlett pears following both early and late picking from the tree and during storage at various temperatures. Pears from regions producing fruit of good shipping quality, and also from regions producing fruit of rather poor shipping quality, were used.

Carbon-dioxide output apparently is an accurate means of measuring the ripening processes in Bartlett pears. The rate of ripening seems to parallel the CO_2 output.

In fruit held at 59° F. (or 15° C.) there is a marked acceleration in the output of carbon dioxide per kilogram of fruit per hour, from the time the fruit is picked until it is soft yellow-ripe. This increase in rate was so great that in certain instances the highest rate of output was seven times the initial rate at the same temperature.

Early-picked fruit respires at a lower initial rate, and this rate is accelerated less rapidly than with later-picked fruit from the same tree. Such fruit requires a longer time after picking before becoming soft and yellow.

Early-picked pears respire more rapidly when soft-ripe than does late-picked fruit from the same trees, and begin to break down and decay more quickly after becoming soft-ripe than does the late-picked fruit.

Pears from regions producing fruit of poor carrying quality have a higher initial CO_2 output, and the rate of CO_2 output increases more rapidly than in fruit with good carrying quality.

At 37° F. there was a slight acceleration in the rate of CO_2 evolution during the one month that fruit was held at this temperature.

At 30° F. there was no acceleration in the rate of CO_2 evolution, even after more than four months in storage.

Upon removing fruit from 30° to 60° F., the initial rate of CO_2 evolution was about equal to what the rate would have been if the fruit had been held at 60° from picking time until a quantity of CO_2 equal to the total amount that had been given off at 30° had been evolved.

Bartlett pears ripen slowly at the lowest temperature possible to hold them without freezing. The length of time they will hold up after removal from cold storage depends entirely upon the amount of ripening that has occurred in the cold-storage rooms. This will depend upon the length of time the fruit has been in storage, and upon the temperature of the storage rooms. Apparently, the fruit will ripen about twice as rapidly at 37° F. as at 30°.

There is an increase in catalase activity in Bartlett pears as they are held in storage after picking, followed by a decrease as the fruit approaches prime eating condition. The decrease in catalase comes much earlier in the life of the fruit than does the decrease in CO_2 output. There seems to be an increase in catalase content for a time after picking, even in pears held at 28° to 33° F.

The concentration of CO_2 is higher and the concentration of oxygen is lower within the tissues of pears held at higher temperatures than in pears held in cold storage. As the fruit ripened at 60° F. there was a sharp increase in carbon-dioxide content and a decrease in oxygen content, with the temperature remaining constant. So the increased respiration rate is not due to better aeration in the riper fruit. The high concentrations of CO_2 and the corresponding low oxygen concentrations in fruit ripening at a temperature about 60° F. appear to be associated with the development of high aroma and flavor in the fruit.

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CALCIUM AND PHOSPHORUS METABOLISM IN DAIRY COWS [1]

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INTRODUCTION

Work done at the agricultural experiment stations of this country in the last 10 years has made it appear that dairy cows under practical conditions are more likely to suffer from a deficiency of assimilable calcium in their rations than from that of any other food constituent. Numerous experiments have been carried out in which the calcium balance has been determined in milking cows. The results point to the conclusion that it is difficult to keep these animals in calcium equilibrium when they are giving more than 5 kilograms of milk daily. The balance has usually been found negative with milk yields above this point, even when the rations contained much more than enough calcium to cover what was being secreted in the milk (*1*, *2*, *3*, *4*).[2]

Extensive experiments have been carried out at the University of Wisconsin in which the calcium balance was determined in milking cows on various diets (*8*, *9*, *10*). It was found that the inclusion of fresh green plant materials in the diet had a highly favorable influence on the rate at which this element was assimilated; and further important observations were made on the values of different kinds of roughage in this respect. According to the Wisconsin investigators, calcium is very poorly assimilated from rations composed of concentrates, timothy hay, and corn silage, even though the calcium content of such rations may be made quite high by the addition of large quantities of bone meal. From rations composed of concentrates, alfalfa hay, and corn silage, but without bone meal, on the other hand, the calcium assimilation is much better, and particularly if the hay used is cured without too long exposure to direct sunlight. Finally, the assimilation of calcium from rations composed of concentrates, fresh green alfalfa, and corn silage is decidedly better than from rations containing the best kind of alfalfa hay. The authors draw the conclusion that calcium assimilation is facilitated when there is present in the rations a vitamin which is contained in fresh green plant material and particularly in the green alfalfa plant. The vitamin in question is destroyed to some extent when the plant material is dried, even under the most favorable conditions. Long exposure to the sun increases the extent to which the vitamin is destroyed, but alfalfa hay which has been exposed in windrows for four days to direct sunlight still contains fairly large amounts of it.

In an earlier investigation Hart and his collaborators studied the effects of cod-liver oil on calcium assimilation in milking goats. They

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[2] Reference is made by number (italic) to "Literature cited," p. 859.

present figures which give some reason for believing that calcium assimilation is facilitated in these animals by the addition of cod-liver oil to the diet (*7*).

The present writers believe that the results above outlined are of such great interest to the science of nutrition in general, and of such great practical importance to the dairy industry, that work along similar lines should be repeated and extended at other experiment stations. Work of this character has been undertaken by the Bureau of Dairying of the United States Department of Agriculture; but in undertaking this work it has been felt that certain fundamental aspects of calcium and phosphorus metabolism and their relation to the technique of balance experiments should be considered more than they often are, and the experiments have been planned from this point of view.

Detailed examination of the figures obtained in past balance experiments seems to show that the rates at which calcium and phosphorus are assimilated are subject to considerable apparently quite capricious variations. The quantities of calcium and phosphorus excreted with the feces of a cow will often vary enough to make large differences in the balances, even though there may be no recorded change in diet or in other conditions to explain these variations. Such irregularities might be due either to real differences in the rate at which absorption of the elements in question took place or to irregularities in the rate at which fecal matter was discharged from the intestinal tract. To the writers it seems probable that both factors play a part. The cow's intestinal tract normally contains a large amount of partially digested material, and the feces are certainly not discharged at an entirely uniform rate. On the other hand, her digestive processes are complicated, involving the voluntary activity of rumination, and it is not at all unlikely that small disturbances of routine might occasionally have considerable effects on these processes, and, through them, on absorption. Some evidence has already been given to indicate that calcium assimilation in cows is particularly affected by disturbances in their routine (*14*), and further results along this line have been obtained in the present investigation.

Besides the irregularities obtained in different periods with the same animals under the same conditions, there are still greater irregularities as between different animals. Hart and his collaborators have suggested that these irregularities may often be caused by differences in the state of the animal's calcium and phosphorus stores. It is an interesting question, therefore, how far a cow is capable of giving up calcium and phosphorus from her body, and what physiological symptoms mark the various stages of depletion. This question also has been kept in view in the present investigation.

Still another question of great physiological interest is how far are calcium and phosphorus metabolism independent of one another. More than 99 per cent of the body's calcium and from 80 to 90 per cent of its phosphorus are contained in the bones (*11, p. 165; 12*). There is strong evidence against the view that the ratio between calcium and phosphorus in bone is subject to considerable variation, and also against the opinion that large changes can take place in either the calcium or the phosphorus content of any of the soft tissues. It is to be expected, therefore, that in the long run the

quantities of calcium and phosphorus gained or lost by an animal would bear the same relation to one another as that which obtains in the bones. For short periods, of course, this relation might be distorted by losses of bone material occurring simultaneously with storage of phosphorus in the soft tissues, or vice versa, and perhaps also by irregularities in the retention of calcium and phosphorus in the intestine. But it is important to distinguish these minor changes from the large and long-continued losses and gains of calcium and phosphorus which can occur through wasting or building up of bone tissue, and this can be done only by following the calcium and phosphorus balances for long periods.

All of the considerations which have been set forth in the preceding paragraphs point to the great advantages to be derived from long-continued determinations of the calcium and phosphorus balances, and the present writers have endeavored to develop a technique which would make possible the carrying out of such experiments.

It was decided to forego the advantages to be derived from a separate collection of urine and feces. In most past experiments on cows the urine and feces have been collected separately by attendants on continuous duty in eight-hour shifts, day and night. This is a very expensive procedure, and experiments in which it is not done can be continued for a much longer time at the same expense. It has seemed to the writers that, in the present state of knowledge, the advantages to be gained from the longer period much more than offset those to be gained from the separation of urine and feces—indeed, the quantities of calcium and phosphorus excreted by cows in their urine are usually such insignificant proportions of the totals that it is not entirely clear why so much trouble and expense has been devoted to separate determination of urinary excretion in previous investigations.

In order to collect the urine and feces together, the writers used apparatus essentially like that devised at the Institute of Animal Nutrition, Pennsylvania State College.[3] The cows are bedded on mattresses of sailcloth stuffed with planer shavings, and behind them are placed funnel-shaped hoppers in which the urine and feces are caught and directed through 4-inch openings into large cylindrical vessels of galvanized iron beneath the floor. The relation between the quantities of urine and feces voided by cows in a 24-hour interval is such that, when the two are stirred together, there results uniform mixture which is easier to stir and handle than the feces alone.

PLAN OF EXPERIMENTS

The work reported in this paper may be regarded as two separate experiments, in each of which two cows were involved, and the calcium, phosphorus, and nitrogen balances determined.

The first experiment (referred to herein as experiment A) was carried out in March and April, 1923. The main objects were to determine whether calcium and phosphorus equilibrium could be maintained in milking cows at different levels of milk yield, and on rations which included alfalfa hay of known origin and history; and also to throw some light on the question of the effects on calcium and

[3] The writers wish to express their indebtedness to J. A. Fries, of the Institute of Animal Nutrition, Pennsylvania State College, for his painstaking kindness and courtesy in demonstrating and explaining this apparatus to one of them.

phosphorus metabolism of various parts of the experimental procedure which is necessary in the carrying out of balance experiments.

The second experiment (referred to herein as experiment B) lasted from early in October, 1923, until late in March, 1924. Its objects were to obtain further information on the manner in which cows can assimilate calcium and phosphorus from alfalfa hay cured in different ways; to observe the effects on calcium and phosphorus metabolism and on milk yield of a long period on a diet deficient in calcium; to get some idea of the extent to which a cow may lose calcium and phosphorus from her body; to throw some light on the question whether the state of the calcium and phosphorus stores has any marked effect on the rate at which these elements are assimilated from a given diet; to observe the effects of cod-liver oil on calcium and phosphorus assimilation in cows; and to gain some idea of the extent to which calcium and phosphorus metabolism are dependent on one another.

In both experiments the quantities of water consumed daily by the cows were determined. Cow 99 consumed from 20 to 30 kilograms daily; cow 54, from 20 to 50 kilograms; and cow 7, from 35 to 55 kilograms. The average daily water consumed was fairly uniform from period to period, and it has not been thought necessary to include tables showing the water consumption in detail.

The amounts of grain and hay consumed in each period are shown in the tables, so that the reader can form some idea as to how the cows' appetites varied throughout the experiments. When any considerable quantities of hay were refused they were analyzed and the results were used in calculating the balances. The hay refused frequently had a composition markedly different from that offered, so that the balances can not be calculated from the quantities of feed consumed. It has not been thought worth while, however, to increase the already rather extensive tabular material by adding the detailed figures for the hay refused and the calculations made therefrom.

ANALYTICAL METHODS

The analytical methods were, in general, with a few minor changes, the same as those described in earlier articles (*13*, *14*).

The writers were impressed with the desirability of a uniform procedure for the determination of moisture. The following experiments illustrate this. Three samples of hay (one of timothy and two of alfalfa) were dried according to different common procedures, namely, for five hours at 100° C., to constant weight at 100° C., and to constant weight at 70° C., in vacuo (25 to 125 mm.), with the following results:

TABLE I.—*Determination of moisture in timothy and alfalfa hay*

Hay	Per cent moisture		
	Five hours at 100° C.	Constant weight at 100° C.	Constant weight at 70° C. in vacuo
Timothy (2 determinations)	5.95 5.95	6.51	5.67
Alfalfa No. 1 (2 determinations)	9.36 9.42	9.62	8.75
Alfalfa No. 2 (2 determinations)	9.01 9.02	9.63	8.34

Heating to constant weight at 70°C. in vacuo was adopted as the most desirable procedure, as loss by decomposition at a higher temperature was regarded as a possibility.

The milk was preserved by adding 2 per cent of a mixture of phenol and alcohol (50 gm. phenol and 10 c. c. 95 per cent alcohol). At first 2 c. c. of preservative per 100 gm. of milk was used; later 2 c. c. of preservative per 100 c. c. milk. The specific gravity of the preservative was 1.057. The specific gravity of the milk with preservative added was determined in each case before samples were taken for analysis. Samples were pipetted.

The urine and feces collected in each 24 hours were thoroughly stirred together, and an aliquot portion was taken out to be mixed with the other aliquots collected during each day of the period. One c. c. of chloroform was added to each aliquot as a preservative. While the period aliquots were being made up the daily aliquots were kept in the moist state in air-tight jars in the ice box. To make sure that there was no loss of nitrogen during this process, the following experiment was carried out: Portions of feces and urine, both separate and mixed, were collected and allowed to stand in the barn for one day. These were then brought into the laboratory and sampled for determination of nitrogen. They were then placed in the ice box and kept for one week and then again sampled for determination of nitrogen. The experiment was carried out in this manner to imitate as nearly as possible the conditions obtaining when the balance experiments were in progress. The results, which showed no loss of nitrogen during storage, follow:

TABLE II.—*Per cent of nitrogen in excreta*

Determination	One day after voiding			Eight days after voiding		
	Urine alone	Feces alone	Feces and urine	Urine alone	Feces alone	Feces and urine
1	0.629	0.454	0.387	0.636	0.466	0.398
2	.618	.452	.381	.629	.462	.368
3	.626	.414	.382	.630	.462	.402
Average	.624	.440	.383	.632	.463	.389

During experiment B the nitrogen in the mixed feces and urine from cow 7 was determined daily for a period of a week, and the total nitrogen for the period obtained by calculation from the daily determinations was compared with that obtained by calculation from determinations made on a composite sample prepared from the daily voidings. The agreement was surprisingly close:

For period calculated from daily determinations..... 2,134.2 gm. N.
For period calculated from composite sample..... 2,129.1 gm. N.

Further information regarding the composition and characteristics of the feeds, the number of analyses made on each feed for the various elements, and the results obtained will be given subsequently.

EXPERIMENT A

The two cows used in this experiment were grade Guernseys. Some of their previous history will be given here, as this may have had some bearing on their calcium and phosphorus metabolism in the experiments described later.

Cow 54 was born January 22, 1915. She had had very little pasture previous to the experiment—51 days in the summer of 1918 and 31 days in the summer of 1919. During most of her life she had received the rations commonly fed at the Beltsville, Md., farm, consisting of grain, alfalfa hay, and corn silage; but from March 12, 1921, until November 17, 1922, she was fed on grain, timothy hay, and corn silage, the grain having ground limestone mixed with it in the proportion of 3 parts to 100. This period without either pasture or legume hay covered the latter part of a lactation period which began September 25, 1920, and ended August 31, 1921, a dry period of about seven weeks, and the whole of the succeeding lactation period beginning October 17, 1921, and ending October 29, 1922. She gave a good deal of milk in this period without legume hay, and there is every reason to think that her calcium and phosphorus stores were considerably depleted.

On November 18, 1922, she was changed to a ration containing alfalfa hay, and received alfalfa hay as a part of her ration continuously until experiment A began. It is doubtful, however, whether she could have made up during this period for the depletion which occurred in the preceding period without legume hay.

She had calved normally on February 8, 1923, and was giving about 19 kilograms of 3.5 per cent milk daily when the experiment started. She weighed 530 kilograms, and was placed in the experimental stall on March 19, 1923.

Cow 99 was born September 25, 1920. She did not have any pasture after the summer of 1921, but her rations contained fair amounts of alfalfa hay all through her prior life. She aborted on November 9, 1922, 109 days before term, and was giving about 7 kilograms of 4.3 per cent milk daily when experiment A started. She weighed 320 kilograms, and was placed in the experimental stall on February 19, 1923.

Both cows received the same amounts and kinds of grain and hay as were fed during the balance experiment for a period of eight days before it started.

The analysis of the urine and feces from both cows was begun on March 21, 1923. On this date cow 99 had been in the experimental stall for about a month, and cow 54 for only two days. The object was to ascertain whether the change to new surroundings would have any disturbing effect on the nitrogen, calcium, and phosphorus metabolism.

In this experiment the urine, feces, and milk were analyzed in four-day periods, aliquots being taken each day and added together for four days.

The feeds consisted of a mixture called grain 50 (see Table III) and alfalfa hay. The hay was second or third cutting alfalfa grown on the Beltsville farm. It had been put in the barn before it had dried quite as much as it should have and heated somewhat while in the barn. Before being used it was chopped up by being put through

the silage cutter and was carefully inspected during this process. Most of it retained its leaves well, but it was brown and there were a good many musty regions scattered through it. After being put through the silage cutter the whole lot (about 600 kilograms) was mixed well and put up in burlap bags.

Four samples were taken from this batch of hay and analyzed for moisture, calcium, phosphorus, and nitrogen. On the basis of these analyses the average calcium, phosphorus, and nitrogen contained in the dry matter of the hay were calculated. Later on, while the hay was being used in the experiment, the moisture contained in it was determined again; and, on the basis of these determinations, the percentages of calcium, phosphorus, and nitrogen in the hay as fed were determined and used in calculating the balances.

Two batches of grain 50 were used in the experiment, No. I and No. II, and the composition of each as fed was determined as described above for the hay. Grain 50 No. I was fed up to and including the first day of Period VIII; grain 50 No. II was fed from the second day of Period VIII on. The composition of the grain and hay as fed is given in Table IV.

The variations in the composition of the feeds and the nature and importance of the errors likely to be introduced by such variations are more fully discussed under the description of the feeds used in experiment B.

It takes a good deal of time for material to pass through a cow's intestinal tract. The feces in any given period are therefore not the residue from the food given in that period, but from the food given some hours previously. A good idea of the way in which this circumstance may distort the calcium balance is given in Tables II, III, and IV of one of Hart's articles (*9*). When the cows were changed from timothy to alfalfa hay the calcium balances during the first week regularly appeared much more positive than they really were, simply because the large amounts of calcium consumed by the cows during the whole period did not begin to be discharged from the intestinal tract until some time after the period had begun. The writers have made a rough calculation of the average length of this intestinal lag from the above-mentioned results and found it to be about two days. In their own experiments, therefore, they have compared the intake of calcium, phosphorus, and nitrogen for each four-day period with the outgo in the last two days of that period and the two following days, endeavoring thus to make some allowance for the intestinal lag. Of course, there are objections to this procedure also. Nitrogen is excreted largely in the urine and with comparatively little lag, and there is also comparatively little lag in the excretion of all three elements in the milk. But the chief interest in these experiments is in the calcium and phosphorus metabolism, and the quantities of these elements excreted in the milk are so much smaller and so much more constant than those excreted in the feces that the above-mentioned method of giving the results seems decidedly the lesser of the two evils.

In Periods V and VI the urine and feces were collected separately by men on continuous duty day and night in eight-hour shifts, in the same manner as in the experiments of Hart and Forbes. This procedure was introduced partly in order to see whether it would have any effect on the assimilation of the three elements studied and

partly to determine the quantities of each element excreted in the urine and feces, respectively, on the diets which were being used.

The experiment was ended for both cows on April 25, 1923. At the end of the experiment cow 54 weighed 575 kilograms and cow 99 333 kilograms. The milk of cow 54 contained 3.8 per cent of fat and that of cow 99, 5 per cent.

The results are given in Tables III to IX, inclusive:

TABLE III.—*Composition of grain mixtures*

Grain 50	Per cent	Grain 55	Per cent
Corn meal	40	Corn meal	30
Wheat bran	30	Wheat bran	20
Cottonseed meal	20	Cottonseed meal	25
Linseed meal *	10	Linseed meal *	25
Salt	1	Salt	1

* Old process.

TABLE IV.—*Composition of the feeds used in experiment A, as fed*

Feed	Water	Ca	P	N
	Per cent	*Per cent*	*Per cent*	*Per cent*
Grain 50 No. I	14.11	0.093	0.763	2.93
Grain 50 No. II	12.97	.105	.782	3.02
Alfalfa hay	12.37	.943	.326	2.45

TABLE V.—*Average daily yield and composition of milk*

Period	Cow 54				Cow 99			
	Yield	Ca	P	N	Yield	Ca	P	N
	Kgm.	*Per cent*	*Per cent*	*Per cent*	*Kgm.*	*Per cent*	*Per cent*	*Per cent*
I	18.50	0.109	0.098	0.495	6.79	0.124	0.107	0.554
II	17.36	.111	.090	.477	6.98	.117	.113	.513
III	17.02	.106	.092	.447	8.05	.118	.115	.572
IV	17.78	.103	.093	.467	7.68	.115	.106	.551
V	17.08	.105	.096	.492	6.86	.115	.112	.539
VI	16.60	.104	.092	.472	7.55	.114	.112	.563
VII	16.46	.106	.090	.477	7.11	.115	.103	.550
VIII	16.45	.104	.094	.475	7.80	.118	.109	.553
IX	15.99	.102	.092	.475	7.54	.114	.107	.566

TABLE VI.—*Average daily quantities, and composition, of separately collected urine and feces voided in Periods V and VI*

Cow	Period	Urine				Feces			
		Quantities voided	Ca	P	N	Quantities voided	Ca	P	N
		Kgm	*Per cent*	*Per cent*	*Per cent*	*Kgm*	*Per cent*	*Per cent*	*Per cent*
Cow 54	V	9.94	0.004	0.012	1.785	22.05	0.225	0.325	0.559
	VI	10.49	.003	.004	1.720	21.68	.224	.328	.556
Cow 99	V	5.12	.001	.042	1.765	9.26	.253	.329	.523
	VI	6.80	.002	.028	1.439	13.17	.233	.291	.525

TABLE VII.—*Daily assimilation of calcium, phosphorus, and nitrogen in relation to feed consumed*

[The "assimilation" is equal to the grams of calcium, phosphorus, and nitrogen in the daily average feed consumed for each period, minus the grams of the same elements contained in the average daily urine and feces]

Cow	Period	Assimilation			Assimilation as percentage of intake			Average daily grain consumed	Average daily hay consumed [a]
		Ca	P	N	Ca	P	N		
								Kgm	*Kgm*
54	I	4.23	11.05	104.5	7.9	12.7	27.0	9.04	5.13
	II	7.07	12.26	80.0	13.0	15.8	22.7	7.71	5.32
	III	9.07	10.75	79.8	18.0	13.0	21.7	8.54	4.95
	IV	9.04	15.59	97.8	16.0	17.9	24.9	9.07	5.20
	V	9.66	14.96	97.9	16.2	17.0	24.6	9.06	5.44
	VI	11.12	16.42	98.9	18.5	18.7	24.7	9.07	5.47
	VII	10.39	18.03	91.1	17.3	20.5	22.8	9.07	5.48
	VIII	10.21	10.48	84.4	17.0	12.5	21.9	8.50	5.49
	IX	10.73	14.19	80.2	17.5	15.9	19.6	9.07	5.49
99	I	10.89	15.37	77.4	29.8	29.1	32.3	5.44	3.25
	II	−4.86	−9.06	−14.6	(b)	(b)	(b)	3.15	1.97
	III	4.32	9.53	51.1	13.1	18.4	22.0	5.41	2.99
	IV	9.87	13.04	54.8	25.4	24.2	23.2	5.44	3.58
	V	1.89	4.95	30.4	7.5	13.2	18.0	3.84	2.33
	VI	4.91	4.66	42.9	13.7	10.4	20.4	4.39	3.29
	VII	−4.06	5.70	35.9	(b)	12.4	16.3	4.78	2.71
	VIII	3.29	6.64	54.6	9.2	13.2	23.6	5.10	3.27
	IX	5.70	8.46	55.0	15.1	15.7	22.2	5.44	3.40

[a] The intake of Ca, P, and N can not be calculated from the figures given in this column, because the hay refused had a different composition from that offered.
[b] Negative.

TABLE VIII.—*Daily calcium, phosphorus, and nitrogen balances of cow 54*

Period	Intake			Outgo in urine and feces			Outgo in milk			Total outgo			Balance		
	Ca	P	N	Ca	P	N	Ca	P	N	Ca	P	N	Ca	P	N
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
I	53.6	86.0	386.7	49.4	76.0	282.2	20.2	18.1	91.6	69.6	94.1	373.8	−16.0	−8.1	+12.9
II	54.3	76.5	353.9	47.2	65.1	274.0	19.3	15.6	82.8	66.5	80.7	356.8	−12.2	−4.2	−2.9
III	50.3	81.5	367.5	41.2	71.7	287.7	18.0	15.7	76.1	59.2	87.4	363.8	−8.9	−5.9	+3.7
IV	56.4	86.1	392.0	47.3	71.6	294.2	18.4	16.5	83.0	65.7	88.1	377.2	−9.3	−2.0	+14.8
V	59.7	86.9	398.7	50.0	72.9	300.7	17.9	16.4	84.0	67.9	89.3	384.7	−8.2	−2.4	+14.0
VI	60.0	87.0	399.8	48.9	71.6	301.0	17.3	15.3	78.3	66.2	86.9	379.3	−6.2	+0.1	+20.5
VII	60.1	87.1	400.1	49.7	70.0	309.0	17.5	14.8	78.5	67.2	84.8	387.5	−7.1	+2.3	+12.6
VIII	60.0	83.2	385.7	49.7	73.5	301.5	17.1	15.5	78.1	66.8	89.0	379.6	−6.8	−5.8	+6.1
IX	61.3	88.8	408.4	50.5	75.0	328.2	16.4	14.7	76.0	66.9	89.7	404.2	−5.6	−0.9	+4.2

TABLE IX.—*Daily calcium, phosphorus, and nitrogen balances of cow 99*

Period	Intake			Outgo in urine and feces			Outgo in milk			Total outgo			Balance		
	Ca	P	N	Ca	P	N	Ca	P	N	Ca	P	N	Ca	P	N
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
I	36.5	52.2	239.8	25.6	37.5	162.4	8.6	7.5	38.6	34.2	45.0	201.0	+2.3	+7.2	+38.8
II	22.4	30.6	142.4	27.2	40.2	157.0	8.2	7.9	35.8	35.4	48.1	192.8	−13.0	−17.5	−50.4
III	33.0	51.2	232.4	28.7	42.3	181.3	9.5	9.3	46.0	38.2	51.6	227.3	−5.2	−0.4	+5.1
IV	38.8	53.2	235.9	28.9	40.8	181.1	8.8	8.1	42.3	37.7	48.9	223.4	+1.1	+4.3	+12.5
V	25.3	37.1	169.2	23.4	32.5	138.7	7.9	7.7	37.0	31.3	40.2	175.7	−6.0	−3.1	−6.5
VI	35.7	44.3	209.9	30.8	40.2	167.0	8.6	8.5	42.5	39.4	48.7	209.5	−3.7	−4.4	+0.4
VII	30.5	45.5	208.3	31.5	40.3	174.4	8.2	7.3	39.1	39.7	47.6	213.5	−9.2	−2.1	−5.2
VIII	35.8	49.9	231.5	32.5	43.7	176.9	9.2	8.5	43.1	41.7	52.2	220.0	−5.9	−2.3	+11.5
IX	37.8	53.7	247.7	32.1	45.4	192.7	8.6	8.1	42.7	40.7	53.5	235.4	−2.9	+0.2	+12.3

DISCUSSION OF EXPERIMENT A

This experiment had two chief objects. The first was to get some idea of how milking cows would assimilate calcium and phosphorus when undistirbed by changes in diet. The second was to determine whether changes in the external surroundings and in the daily routine of cows would have any effect on the calcium and phosphorus assimilation. Some information on both of these questions is absolutely necessary before balance experiments can be intelligently planned or interpreted. But it can readily be seen also that each of them is a large problem in itself, which would require a great deal of experimentation for a complete solution.

In studying the results of experiment A the writers were anxious to get some measure of the efficiency with which calcium and phosphorus are absorbed from the intestinal tract. The balances are not the best measure of this in milking cows, because they are affected by the quantities of the two elements lost in the milk. As a measure of the efficiency of intestinal absorption it was decided to take the difference between the total intake of each element under consideration and the quantity lost in the feces and urine. In the case of nitrogen the figures for these differences are not of much value as an index of intestinal absorption, because large quantities of this element are lost in the urine. But the quantities of calcium and phosphorus lost in the urine of cows on the diet used in this experiment are comparatively small, as is seen in Table VI, and the difference between the total intake and the output in the urine and feces therefore comes close to representing the net intestinal absorption in the case of these elements. The writers call this difference "assimilation" of the elements in question.

In Table VII the assimilation of the various elements by the two cows is given, as is also the assimilation as percentage of the intake and the feed consumed in the different periods. A study of the table shows that things went much more smoothly with cow 54 than with cow 99. Cow 54 was the older and more phlegmatic of the two. She was not at any time seriously off feed and after the first two periods her assimilation of all three of the elements studied was fairly constant. Cow 99, on the other hand, was very badly off feed in Period II, and noticeably so in Periods V, VI, and VII. Her assimilation of all three of the elements studied, but particularly of calcium, was noticeably reduced in the periods when she was off feed.

The manner in which the cows were treated has already been noted. Consideration was given to the question whether there was any close connection between changes in their routine, loss of appetite, and changes in the assimilation of calcium, phosphorus, and nitrogen, and it was found that the periods of loss of appetite sometimes did and sometimes did not coincide with the changes in routine.

The results point rather strongly to the view, however, that either changes in routine or digestive disturbances which result in even slight loss of appetite may markedly reduce the calcium assimilation. This process was very noticeably retarded in cow 54 in the first two periods just after she was put in the experimental stall. In the case of cow 99 it became negative in the second and seventh periods and was markedly retarded in the fifth period, in all three of which she was more or less off feed. The hand collection of urine and feces had no

effect on either the appetite or calcium assimilation of cow 54, while it apparently had a considerable effect on both in the case of cow 99. It may be, of course, that this was merely a coincidence, but it does not seem improbable that a procedure of this type might fail to produce any effect on a phlegmatic animal, and might, nevertheless, have a very considerable effect on a more nervous one.

From the results which have been obtained it seems safe to draw the conclusion that various kinds of disturbance may have very considerable effects in retarding calcium assimilation. It is not to be expected that such results would be very uniform or constant. But it is clear that the results of balance experiments will be more valuable the longer they are continued and the less the animals used in them suffer from digestive and other disturbances.

From Tables VIII and IX it may be seen that the calcium balances were always negative in the case of cow 54, and usually negative in the case of cow 99 also, in spite of the fact that she was not giving very much milk. The phosphorus balances also were generally negative in both cows. Cow 54 had a decidedly positive nitrogen balance for the whole experiment, and cow 99 a slightly positive one.

EXPERIMENT B

Two cows were used in the experiment, namely cow 7 and cow 54 Cow 54 was the same animal that had been used in experiment A Most of the essential points in her previous history have already been given, and it is only necessary to add that from April 23, 1923, when she came off experiment A, until October 6, 1923, when she went on experiment B, she was milking and on a ration which contained alfalfa hay, but without pasture. She lost considerable amounts of calcium and phosphorus from her body in the course of experiment A, and it is unlikely that she made up these losses in the period which intervened between the end of that experiment and the beginning of experiment B. It is probable, therefore, that at the beginning of experiment B her calcium and phosphorus stores were considerably depleted. From September 25, 1923, until October 6, when experiment B started, she received grain 50 and alfalfa hay in the same amounts as were fed during the first six weeks of the experiment.

Cow 7 was a grade Holstein born in the autumn of 1919. During the first two and a half years of her life she was on a farm near Washington, D. C., where she had pasture in the summer. She was purchased for the Beltsville farm on March 27, 1922, soon after calving. From that date until she went dry on June 21, 1923, she received a ration containing liberal amounts of alfalfa hay with grain and silage in addition, but no pasture. On June 22, 1923, she was turned out on pasture, and remained on pasture, with some grain in addition, until she calved on August 13, 1923. From that date she received liberal amounts of alfalfa hay and grain until the experiment began. From September 25, 1923, until the experiment began on October 6, she received grain and alfalfa hay in the same quantities as were fed during the first six weeks of the balance experiment.

The feeds used in experiment B were two grain mixtures, two grades of alfalfa hay, and timothy hay. When the roughage consisted of alfalfa hay, grain mixture 50 was used; with timothy as roughage, grain mixture 55 was fed (see Table III). In Periods XXI, XXII,

and XXIII cod-liver oil was added to the rations. Table XII shows the nature and composition of the food used in the different periods of the experiment. The quantities of hay and grain consumed in the different periods are given in Tables XIV and XV.

The hay which is called "good alfalfa" in the tables and text had been cut in the morning on a favorable sunny day in June and was taken in during the afternoon and cured on racks under a shed in such a way that the air could get through it without its being exposed to the sun. It was quite green when the curing had been finished. The hay called "poor alfalfa" had been allowed to lie in the field in windrows for nine days and had been rained on during most of two days. It had turned brown when it dried. Both lots of hay were put through the silage cutter and each was then mixed and stored in burlap sacks.

Two batches of timothy hay, which are designated timothy No. I and timothy No. II, respectively, were used during the experiment, each batch being put through the silage cutter, mixed, and sampled for analysis separately. Of grain 50, three separate batches, and of grain 55, two separate batches were used. These are designated grain 50, Nos. I, II, and III, and grain 55, Nos. I and II, respectively. The periods in which the various batches of feed were used are given in Table XII.

The same general procedure was used in obtaining figures for the composition of the various feeds as that described in regard to the feeds of experiment A.

The writers were impressed by the size of the variations in the mineral content of the different batches of feed, and even within the same batch, in the case of the hay, in spite of the precaution of passing each batch of hay through the silage cutter and mixing it before storing it in bags. They consider it worth while to give in some detail the results of their experience in this matter in order to make clear how far their own results and those of other investigators may be taken as really representing the metabolic processes of the animals studied and how far the results are likely to be influenced by errors in the analysis and sampling of the feeds. In order to throw light on this question the writers give in detail the procedure and analytical results with the batch of grain in which the variations in phosphorus content were greatest, and with the batch of hay in which the variations in calcium content were greatest. The calcium content of the grain and the phosphorus content of the hay would have comparatively little influence on the results. Later on, the average figures for all the other batches of feed will be given, and from them it can be judged how great the variations are likely to be when large samples of feed are compared with one another.

The "good alfalfa" showed greater variations in calcium content than any of the other batches of hay. After this hay was cured it was put through the silage cutter, and the whole batch was then spread out on a cement floor and mixed. Two samples (designated I and II in Table X) were taken from the hay while it lay on the floor, and the rest was then put in burlap sacks. A third sample (designated III in Table X) was collected from three of the sacks. These three samples were then ground rather coarsely in a small meat cutter, and analyzed. The complete figures for the calcium analyses made on them are given in Table X.

The agreement among these analyses was not considered satisfactory, and three more large samples were taken, each of these being collected from three different sacks. These samples were ground to a fineness of 20 mesh in an electrically driven mill and were then analyzed. The figures for these analyses are given in Table X under the designations IV, V, and VI. As will be seen in the table, the agreement between the duplicates was more satisfactory than in I, II, and III, but there is a difference of more than 10 per cent between the average figures for V and VI, and the average for the large samples I, II, and III also differs by about 10 per cent from that for IV, V, and VI. The effect which differences of this order would have on the figures calculated for the balances are discussed later.

As the average figure for the whole batch of good alfalfa hay 2.574 was used. The manner in which it was calculated from the individual figures is shown in Table X.

Grain 50, No. I, showed a greater variation in phosphorus than any of the other batches of grain. It and all the other batches were mixed on a cement floor and large samples were taken out and reground for analysis. The individual results for grain 50, No. I, are given in Table XI. The agreement is much more satisfactory than in the case of the hay. But, as will be seen in Table XII, there is a difference of about 20 per cent between the average phosphorus of grain 50, No. II, and that of grain 50, No. III.

The periods for which individual analyses of the milk and excreta were made in experiment B were seven days instead of only four, as in experiment A. The writers continued, however, as in experiment A, to allow for two days' lag in the fecal excretion, by which is meant that, in calculating the balances, they subtract the quantities of calcium, phosphorus, and nitrogen excreted in the urine, feces, and milk for the period of, for example, March 3 to 9, inclusive, from the quantities of the same elements consumed in the feed in the period from March 1 to 7, inclusive. The results are given in detail in Tables X to XVII, inclusive.

TABLE X.—*Calcium content of the "good alfalfa" hay used in experiment B*

[The calcium content of this varied more than that of any of the other batches of hay used in this experiment. The Roman numerals indicate the large samples taken, and the Arabic numerals the several analyses made on each of these. The figures show the percentage of Ca contained in the dry matter of the hay]

Analysis No.	Samples					
	I	II	III	IV	V	VI
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	2.530	2.834	2.820	2.510	2.311	2.560
2	2.556	2.887	2.724	2.508	2.325	2.597
3	2.423	2.755	2.598			
Average	2.503	2.825	2.714	2.509	2.318	2.578

	Per cent
Average for Samples I, II, and III	2.681
Average for Samples IV, V, and VI	2.468
Average for whole batch, giving same weight to Samples IV, V, and VI, as to Samples I, II, and III	2.574

TABLE XI.—*Phosphorus content of grain 50, No. I*

[The phosphorus content of this batch of grain varied more than that of any of the other batches used in experiment B. The figures show the percentages of phosphorus contained in the dry matter of the grain]

Analysis No.	Sample I	Sample II
	Per cent	*Per cent*
1	0. 930	0. 967
2	. 899	. 920
3	. 929	. 921
Average	. 919	. 936

	Per cent
Average for batch	0. 927

TABLE XII.—*Composition of the batches of feed used in the different periods of experiment B*

HAY

Periods of experiment	Batch of feed used	Moisture in batch as fed	Ca, P, & N in batch as fed		
			Ca	P	N
		Per cent	*Per cent*	*Per cent*	*Per cent*
I and II	Good alfalfa	11. 88	2. 268	0. 169	2. 097
III and IV	do	11. 87	2. 268	. 169	2. 097
V and VI	Poor alfalfa	12. 70	1. 924	. 172	2. 003
VII–IX	Timothy No. I	11. 97	. 321	. 124	. 821
X–XII	do	13. 07	. 317	. 123	. 811
XIII–XVII	Timothy No. II	12. 17	. 256	. 101	. 733
XVIII–XXIII	Poor alfalfa	12. 63	1. 926	. 172	2. 004
XXIV and XXV	Good alfalfa	12. 09	2. 263	. 169	2. 092
	GRAIN				
I and II	Grain 50, No. I	12. 56	0. 111	0. 811	3. 043
III and IV	do	12. 84	. 111	. 808	3. 034
V and VI	Grain 50, No. II	12. 19	. 129	. 794	3. 072
VII–IX	Grain 55, No. I	12. 02	. 162	. 747	3. 755
X–XII	do	12. 52	. 161	. 743	3. 740
XIII–XV	do	12. 05	. 162	. 747	3. 760
XVI and XVII	Grain 55, No. II	11. 13	. 156	. 779	3. 790
XVIII–XXI first 5 days	Grain 50, No. II	12. 74	. 128	. 789	3. 053
XXI last two days; XXV first 5 days	Grain 50, No. III	12. 34	. 116	. 952	3. 206
XXV last 2 days	Grain 50, No. II	12. 88	. 128	. 788	3. 047

TABLE XIII.—*Average daily yield and composition of milk*

Period	Cow 7				Cow 54			
	Yield	Ca	P	N	Yield	Ca	P	N
	Kgm.	*P. ct.*	*P. ct.*	*P. ct.*	*Kgm.*	*P. ct.*	*P. ct.*	*P. ct.*
I	15. 96	0. 124	0. 112	0. 555	5. 26	0. 098	0. 085	0. 537
II	15. 67	. 126	. 111	. 551	5. 09	. 098	. 085	. 534
III	15. 89	. 128	. 112	. 564	5. 01	. 095	. 083	. 527
IV	15. 34	. 132	. 115	. 571	4. 86	. 099	. 083	. 530
V	14. 36	. 132	. 111	. 585	4. 50	. 099	. 083	. 532
VI	14. 37	. 134	. 115	. 591	4. 24	. 099	. 083	. 531
VII	14. 39	. 129	. 110	. 581	3. 87	. 100	. 084	. 532
VIII	13. 11	. 130	. 111	. 604	3. 34	. 096	. 081	. 547
IX	12. 37	. 130	. 115	. 588	2. 97	. 087	. 083	. 538
X	9. 87	. 139	. 117	. 594	2. 79	. 094	. 081	. 531
XI	12. 44	. 131	. 116	. 625	2. 63	. 093	. 079	. 523
XII	11. 80	. 131	. 112	. 618	2. 39	. 087	. 078	. 527
XIII	11. 47	. 134	. 114	. 645	2. 14	. 089	. 079	. 539
XIV	10. 57	. 126	. 112	. 636	1. 80	. 093	. 085	. 557
XV	10. 14	. 129	. 111	. 640	1. 77	. 093	. 084	. 539
XVI	10. 29	. 130	. 113	. 643	1. 71	. 097	. 088	. 566
XVII	9. 77	. 128	. 113	. 636	1. 53	. 104	. 090	. 582
XVIII	10. 37	. 135	. 119	. 634	1. 16	. 109	. 094	. 600
XIX	10. 37	. 133	. 116	. 637	0. 84	. 114	. 090	. 641
XX	10. 11	. 135	. 113	. 655	. 21	. 101	. 078	. 813
XXI	10. 69	. 132	. 111	. 639	. 00			
XXII	11. 10	. 130	. 108	. 607	. 00			
XXIII	9. 83	. 135	. 107	. 656	. 00			
XXIV	10. 49	. 145	. 123	. 649	. 00			
XXV	10. 81	. 137	. 118	. 636	. 00			

TABLE XIV.—*Daily assimilation of calcium, phosphorus, and nitrogen in relation to intake and to feed consumed, cow 7*

Ration	Period	Assimilation			Assimilation as per cent of intake			Average daily grain consumed	Average daily hay consumed [a]
		Ca	P	N	Ca	P	N		
		Gm.	*Gm.*	*Gm.*	*P. ct.*	*P. ct.*	*P. ct.*	*Kgm.*	*Kgm.*
Grain 50, good alfalfa hay	I	17.0	12.9	104.0	9.4	16.6	25.7	8.00	7.59
	II	12.9	14.0	95.1	7.3	18.1	23.8	7.94	7.37
	III	21.3	14.4	98.0	11.5	18.5	24.1	8.00	7.88
	IV	17.9	16.3	94.2	9.8	21.0	23.3	8.00	7.77
Grain 50, poor alfalfa hay	V	4.0	17.1	77.5	2.6	22.2	19.6	8.00	7.69
	VI	3.4	12.3	73.8	2.3	16.2	18.9	8.00	7.45
	VII	−.1	10.8	90.0	(b)	15.5	24.6	8.00	7.93
	VIII	6.9	11.0	86.6	18.3	15.9	23.8	8.00	7.89
	IX	9.3	13.1	87.9	26.7	19.1	24.5	8.00	7.48
	X	1.9	−.7	30.9	7.4	(b)	13.1	5.14	5.59
Grain 55, timothy hay	XI	6.1	7.5	73.5	16.5	10.9	20.4	8.00	7.80
	XII	9.9	9.5	77.7	27.3	13.8	21.6	8.00	7.70
	XIII	10.0	9.0	79.2	31.7	13.4	22.4	8.00	7.41
	XIV	11.0	.3	60.8	36.1	.4	17.3	8.00	6.90
	XV	11.7	6.2	69.3	41.1	9.4	20.1	8.00	6.13
	XVI	5.3	6.2	56.1	18.4	9.1	16.1	8.00	6.38
	XVII	2.9	12.6	70.0	10.3	18.4	20.1	8.00	6.20
	XVIII	14.9	13.9	57.2	16.0	19.7	17.3	8.00	4.49
Grain 50, poor alfalfa hay	XIX	8.1	16.9	72.1	6.5	23.0	19.8	8.00	6.01
	XX	−.96	12.2	66.3	(b)	16.3	17.5	8.00	6.84
Grain 50, poor alfalfa hay, Cod-liver oil	XXI	2.2	9.2	66.7	1.7	11.8	17.9	8.00	6.32
	XXII	−1.8	11.7	69.7	(b)	13.5	18.5	8.00	6.06
	XXIII	2.2	17.9	65.6	2.1	21.1	18.3	8.00	5.11
Grain 50, good alfalfa hay	XXIV	16.9	14.8	78.6	13.8	17.5	21.8	8.00	5.00
	XXV	16.4	15.0	90.9	13.3	18.6	25.4	8.00	5.00

[a] The intake of Ca, P, and N can not be calculated from the figures given in this column, because the hay refused had a different composition from that offered.
[b] Negative.

TABLE XV.—*Daily assimilation of calcium, phosphorus, and nitrogen in relation to intake and to feed consumed, cow 54*

Ration	Period	Assimilation			Assimilation as per cent of intake			Average daily grain consumed	Average daily hay consumed [a]
		Ca	P	N	Ca	P	N		
		Gm.	*Gm.*	*Gm.*	*P. ct.*	*P. ct.*	*P. ct.*	*Kgm.*	*Kgm.*
Grain 50, good alfalfa hay	I	3.9	1.5	53.4	3.3	2.2	16.8	7.00	4.95
	II	−1.6	1.6	40.8	(b)	2.5	13.0	7.00	4.69
	III	1.8	3.5	31.9	1.5	5.5	10.1	7.00	4.97
	IV	2.5	.5	32.0	2.1	.7	10.1	7.00	4.97
Grain 50, poor alfalfa hay	V	−5.8	3.9	17.6	(b)	6.1	5.7	7.00	4.74
	VI	−5.6	.2	16.5	(b)	.4	5.4	7.00	4.75
	VII	−12.3	−1.7	25.2	(b)	(b)	8.3	7.00	4.94
	VIII	.6	−.2	30.4	2.3	(b)	10.1	7.00	4.77
	IX	−1.3	−.3	27.9	(b)	(b)	9.2	7.00	4.94
	X	−3.3	1.4	17.4	(b)	2.4	5.8	7.00	4.94
Grain 55, timothy hay	XI	−.8	.6	17.4	(b)	1.0	5.8	7.00	4.86
	XII	.2	−2.5	17.6	.8	(b)	6.0	7.00	4.38
	XIII	.5	−2.5	11.2	2.5	(b)	3.9	7.00	3.48
	XIV	2.4	−1.7	18.9	11.5	(b)	6.5	6.99	3.78
	XV	1.5	−.2	12.8	8.0	(b)	4.8	6.40	3.28
	XVI	−.8	−1.7	15.5	(b)	(b)	6.1	6.00	4.01
	XVII	−1.3	5.0	31.7	(b)	10.0	12.6	6.00	3.54
	XVIII	−.2	3.1	1.8	(b)	5.9	.8	6.00	2.74
Grain 50, poor alfalfa hay	XIX	2.1	2.1	19.0	2.5	3.9	7.2	6.00	4.04
	XX	−7.5	−1.0	3.7	(b)	(b)	1.5	6.00	3.47
Grain 50, poor alfalfa hay, Cod-liver oil	XXI	−4.4	.2	9.9	(b)	.3	3.8	6.14	3.37
	XXII	−9.9	−.9	.9	(b)	(b)	.3	6.00	3.28
	XXIII	−8.2	−.7	3.2	(b)	(b)	1.2	6.00	3.49
Grain 50, good alfalfa hay	XXIV	2.3	3.1	12.5	3.0	4.9	4.9	6.00	3.00
	XXV	1.4	2.3	20.2	1.8	3.8	8.0	6.00	3.00

[a] The intake of Ca, P, and N can not be calculated from the figures given in this column, because the hay refused had a different composition from that offered.
[b] Negative.

TABLE XVI.—*Daily calcium, phosphorus, and nitrogen balances of cow 7*

Ration	Period	Intake			Outgo in urine and feces			Outgo in milk			Total outgo			Balance		
		Ca	P	N	Ca	P	N	Ca	P	N	Ca	P	N	Ca	P	N
		Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Grain 50, good alfalfa hay	I	181.0	77.8	404.0	164.0	64.9	300.0	19.8	17.9	88.6	183.8	82.8	388.6	−2.8	−5.0	+15.4
	II	176.4	77.0	399.3	163.5	63.1	304.2	19.7	17.4	86.3	183.2	80.5	390.5	−6.8	−3.5	+8.8
	III	186.2	77.9	407.3	164.9	63.5	309.3	20.3	17.8	89.6	185.2	81.3	398.9	+1.0	−3.4	+8.4
	IV	182.3	77.6	404.4	163.0	61.3	310.2	20.3	17.6	87.6	183.3	78.9	397.8	−1.0	−1.3	+6.6
Grain 50, poor alfalfa hay	V	153.9	77.3	395.3	149.8	60.2	317.8	19.0	15.9	84.0	168.8	76.1	401.8	−14.9	+1.2	−6.5
	VI	148.0	76.1	391.2	144.7	63.8	317.4	19.3	16.5	84.9	164.0	80.3	402.3	−16.0	−4.2	−11.1
Grain 55, timothy hay	VII	38.1	69.5	365.3	38.2	58.7	275.3	18.6	15.8	83.6	56.8	74.5	358.9	−18.7	−5.0	+6.4
	VIII	37.8	69.4	364.6	30.9	58.4	278.0	17.0	14.6	79.2	47.9	73.0	357.2	−10.1	−3.6	+7.4
	IX	34.8	68.6	358.1	25.5	55.5	270.2	16.1	14.2	72.7	41.6	69.7	342.9	−6.8	−1.1	+15.2
	X	25.6	45.0	236.7	23.7	45.7	207.8	13.7	11.5	58.6	37.4	57.2	266.4	−11.8	−12.2	−29.7
	XI	37.0	68.9	360.9	30.9	61.4	287.4	16.3	14.4	77.8	47.2	75.8	365.2	−10.2	−6.9	−4.3
	XII	36.3	68.7	359.3	26.4	59.2	281.6	15.5	13.2	72.9	41.9	72.4	354.5	−5.6	−3.7	+4.8
	XIII	31.5	67.0	353.0	21.5	58.0	273.8	15.4	13.1	74.0	36.9	71.1	347.8	−5.4	−4.1	+5.2
	XIV	30.5	66.7	351.0	19.5	66.4	290.2	13.3	11.8	67.2	32.8	78.2	357.4	−2.3	−11.5	−6.4
	XV	28.5	65.9	345.2	16.8	59.7	275.9	13.1	11.3	64.9	29.9	71.0	340.8	−1.4	−5.1	+4.4
	XVI	28.8	67.9	348.5	23.5	61.7	292.4	13.4	11.6	66.1	36.9	73.3	358.5	−8.1	−5.4	−10.0
	XVII	28.2	68.5	347.4	25.3	55.9	277.4	12.5	11.0	62.1	37.8	66.9	339.5	−9.6	+1.6	+7.9
Grain 50, poor alfalfa hay	XVIII	93.1	70.6	331.5	78.2	56.6	274.3	14.0	12.4	65.8	92.2	69.0	340.1	+.9	+1.6	−8.6
	XIX	124.7	73.4	363.3	116.6	56.5	291.2	13.8	12.0	66.2	130.4	68.5	357.4	−5.7	+4.9	+5.9
	XX	140.5	74.8	379.8	141.4	62.6	313.5	13.7	11.4	66.2	155.1	74.0	379.7	−14.6	+.8	+.1
Grain 50, poor alfalfa hay *	XXI	129.9	77.5	372.7	127.7	68.3	306.0	14.1	11.9	68.3	141.8	80.2	374.3	−11.9	−2.7	−1.6
	XXII	124.8	86.5	377.0	126.6	74.8	307.3	14.4	12.0	67.4	141.0	86.8	374.7	−16.2	−.3	+2.3
	XXIII	106.7	84.8	358.2	104.5	66.9	292.6	13.3	10.5	64.5	117.8	77.4	357.1	−11.1	+7.4	+1.1
Grain 50, good alfalfa hay	XXIV	122.4	84.6	361.3	105.6	69.8	282.7	15.2	12.9	68.1	120.8	82.7	350.8	+1.6	+1.9	+10.5
	XXV	122.7	80.9	357.7	106.3	65.9	266.8	14.8	12.8	68.8	121.1	78.7	335.6	+1.6	+2.2	+22.1

* With cod-liver oil.

TABLE XVII.—*Daily calcium, phosphorus, and nitrogen balances of cow 54*

Ration	Period	Intake			Outgo in urine and feces			Outgo in milk			Total outgo			Balance		
		Ca	P	N	Ca	P	N	Ca	P	N	Ca	P	N	Ca	P	N
		Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Grain 50, good alfalfa hay	I	120.4	65.2	317.1	116.5	63.7	263.7	5.2	4.5	28.2	121.7	68.2	291.9	−1.3	−3.0	+25.2
	II	116.3	64.9	313.3	117.9	63.2	272.5	5.0	4.3	27.2	122.9	67.5	299.7	−6.6	−2.6	+13.6
	III	120.6	65.0	316.5	118.8	61.4	284.6	4.8	4.2	26.4	123.6	65.6	311.0	−3.0	−.6	+5.5
	IV	120.5	65.0	316.5	118.0	64.5	284.5	4.8	4.0	25.7	122.8	68.5	310.2	−2.3	−3.5	+6.3
Grain 50, poor alfalfa hay	V	97.0	64.2	307.8	102.8	60.3	290.2	4.5	3.7	23.9	107.3	64.0	314.1	−10.3	+.2	−6.3
	VI	98.1	63.6	308.4	103.7	63.4	291.9	4.2	3.5	22.5	107.9	66.9	314.4	−9.8	−3.3	−6.0
Grain 55, timothy hay	VII	27.1	58.4	303.6	39.4	60.1	278.4	3.9	3.3	20.6	43.3	63.4	299.0	−16.2	−5.0	+4.6
	VIII	26.4	58.1	301.7	25.8	58.3	271.3	3.2	2.7	18.3	29.0	61.0	289.6	−2.6	−2.9	+12.1
	IX	27.1	58.4	303.6	28.4	58.7	275.7	2.6	2.5	16.0	31.0	61.2	291.7	−3.9	−2.8	+11.9
	X	26.9	58.1	301.7	30.2	56.7	284.3	2.6	2.3	14.8	32.8	59.0	299.1	−5.9	−.9	+2.6
	XI	26.5	57.9	300.8	27.3	57.3	283.4	2.4	2.1	13.7	29.7	59.4	297.1	−3.2	−1.5	+3.7
	XII	24.5	57.1	295.6	24.3	59.6	278.0	2.1	1.9	12.6	26.4	61.5	290.6	−1.9	+4.4	+5.0
	XIII	19.9	55.6	287.1	19.4	58.1	275.9	1.9	1.7	11.6	21.3	59.8	287.5	−1.4	+4.2	−.4
	XIV	20.9	55.9	289.7	18.5	57.6	270.8	1.8	1.7	10.8	20.3	59.3	281.6	+.6	−3.4	+8.1
	XV	18.7	51.0	264.2	17.2	51.2	251.4	1.6	1.5	9.5	18.8	52.7	260.9	−.1	−1.7	+3.3
	XVI	19.6	48.8	255.9	20.4	50.5	240.4	1.7	1.5	9.7	22.1	52.0	250.1	−2.5	−3.2	+5.8
	XVII	18.2	50.1	251.7	19.5	45.1	220.0	1.6	1.4	8.9	21.1	46.5	228.9	−2.9	+3.6	+22.8
Grain 50, poor alfalfa hay	XVIII	59.8	51.9	236.9	60.0	48.8	235.1	1.3	1.1	6.9	61.3	49.9	242.0	−1.5	+2.0	−5.1
	XIX	85.2	54.2	263.5	83.1	52.1	244.5	1.0	.8	5.4	84.1	52.9	249.9	+1.1	+1.3	+13.6
	XX	73.1	53.0	250.6	80.7	53.9	246.9	.2	.2	1.7	80.9	54.1	248.6	−7.8	−1.1	+2.0
Grain 50, poor alfalfa hay, cod-liver oil	XXI	77.1	56.8	261.5	81.5	56.6	251.6	.0	.0	.0	81.5	56.6	251.6	−4.4	+.2	+9.9
	XXII	70.6	62.8	258.6	80.5	63.7	257.7	.0	.0	.0	80.5	63.7	257.7	−9.9	−.9	+.9
	XXIII	74.1	63.1	262.3	82.4	63.8	259.1	.0	.0	.0	82.4	63.8	259.1	−8.3	−.7	+3.2
Grain 50, good alfalfa hay	XXIV	74.8	62.2	255.3	72.6	59.1	242.8	.0	.0	.0	72.6	59.1	242.8	+2.2	+3.1	+12.5
	XXV	75.0	60.1	253.2	73.6	57.8	233.0	.0	.0	.0	73.6	57.8	233.0	+1.4	+2.3	+20.2

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TABLE XVIII.—*The calcium assimilation of dairy cows, in relation to various factors which may influence it*

[The data given in this table are based on the experimental work reported in this article and on that reported in four articles by Hart and collaborators (*7, 8, 9, 10*)]

Where experimental work was done and reported [a]	Cow, experiment, and period Nos.	Days in period	Ca assimilation as percentage of intake	Ca intake, grams daily	Ca balance, grams daily	P balance, grams daily	Ca/P in intake	Milk yield, kilograms daily	Character of diet	Average percentage calcium assimilation for group
Wisconsin (*10*) [b]	Animal 1	14	43.9	49.68	−7.02	+7.23	0.61	22.91	Lawn grass	33.9
Wisconsin (*10*)	Animal 4	14	38.6	57.54	−5.33	+3.10	.84	21.29		
Wisconsin (*10*)	Animal 2	14	35.8	49.68	−13.29	+2.25	.61	23.80		
Wisconsin (*10*)	Animal 6	14	31.3	52.52	−2.34	+2.03	1.06	14.45		
Wisconsin (*10*)	Animal 5	14	20.1	53.52	−2.74	+1.86	1.08	8.98		
Wisconsin (*8*)	Animal 1	21	39.9	89.66	+28.91	+12.77	1.87	4.19	Green alfalfa	32.3
Wisconsin (*8*)	Animal 2	21	29.7	95.05	+8.61	+5.86	1.48	15.33		
Wisconsin (*8*)	Animal 3	21	27.3	93.35	+14.46	+5.41	1.75	8.52		
Wisconsin (*8*)	Animal 1	21	31.4	79.30	+9.09	+7.48	1.72	8.86	Alfalfa hay cured under caps	27.7
Wisconsin (*8*)	Animal 2	21	31.2	82.05	+3.19	+4.08	1.33	18.52		
Wisconsin (*8*)	Animal 3	21	20.5	80.20	+2.71	+3.56	1.58	11.19		
Wisconsin (*9*)	Animal 1	21	38.7	28.02	−16.41	−14.94	.60	21.41	Timothy hay unsupplemented.	22.1
Wisconsin (*9*)	Animal 2	21	20.5	24.80	−9.52	−7.57	.72	10.38		
Wisconsin (*9*)	Animal 3	21	7.1	27.58	−21.00	−16.22	.62	17.67		
Wisconsin (*9*)	Animal 1	21	21.7	95.46	−6.49	−3.60	2.06	20.37	Alfalfa hay cured in windrows.	18.8
Wisconsin (*9*)	Animal 3	21	19.7	95.13	−2.36	+.16	2.16	16.22		
Wisconsin (*9*)	Animal 2	21	15.0	92.22	−2.31	−2.71	2.80	10.50		
Wisconsin (*10*)	Animal 3	14	22.1	100.60	+5.76	+3.62	1.21	20.74	Lawn grass plus bone meal	18.3
Wisconsin (*10*)	Animal 1	14	21.4	117.71	+.47	+.27	1.14	20.28		
Wisconsin (*10*)	Animal 6	14	20.2	112.06	+6.59	+1.54	1.51	11.60		
Wisconsin (*10*)	Animal 4	14	19.3	115.76	−1.86	+4.73	1.30	17.49		
Wisconsin (*10*)	Animal 5	14	15.7	112.06	+4.75	+3.43	1.51	8.20		
Wisconsin (*10*)	Animal 2	14	11.0	124.43	−13.11	−5.24	1.15	22.42		
Beltsville [c]	Cow 54, experiment A	28	17.2	58.25	−7.46	−2.07	.68	16.77	Alfalfa hay which had heated in barn.	14.5
Beltsville	Cow 99, experiment A	36	11.8	32.88	−4.73	−2.01	.71	7.40		
Beltsville	Cow 7, experiment B, Periods VIII–XVII	70	23.5	31.91	−7.11	−5.23	.49	11.18	Timothy hay	13.3
Beltsville	Cow 54, experiment B, Periods VIII–XVII	70	(*d*)	22.88	−2.38	−2.12	.42	2.31		
Wisconsin (*9*)	Animal 3	28	11.3	96.30	−7.89	−10.71	1.27	14.37	Timothy hay plus bone meal	10.0
Wisconsin (*9*)	Animal 1	28	10.9	96.74	−13.22	−9.76	1.24	18.34		
Wisconsin (*9*)	Animal 2	21	7.8	92.63	−5.06	−11.00	1.46	8.21		
Beltsville	Cow 7, experiment B, Periods XXIV–XXV	14	13.6	122.55	+1.60	+2.05	1.48	10.65	Good alfalfa hay	8.0
Beltsville	Cow 54, experiment B, Periods XXIV–XXV	14	2.4	74.90	+1.80	+2.70	1.22	0		
Beltsville	Cow 7, experiment B, Periods I–IV	28	9.5	181.48	−2.40	−3.30	2.34	15.71	Good alfalfa hay	5.7
Beltsville	Cow 54, experiment B, Periods I–IV	28	1.8	119.95	−2.80	−2.20	1.84	5.05		
Beltsville	Cow 7, experiment B, Periods V–VI	14	2.4	150.95	−15.45	−1.50	1.97	14.36	Poor alfalfa hay	(*d*)
Beltsville	Cow 54, experiment B, Periods V–VI	14	(*d*)	97.55	−10.05	−1.55	1.53	4.37		
Beltsville	Cow 7, experiment B, Periods XIX–XXIII	35	1.6	125.32	−11.90	+2.02	1.58	10.42	Poor alfalfa hay	(*d*)
Beltsville	Cow 54, experiment B, Periods XIX–XXIII	35	(*d*)	76.02	−5.86	−.24	1.31	.21		

[a] The figures in italics in the first column refer to "Literature cited" at end of article.
[b] Wisconsin Agricultural Experiment Station, University of Wisconsin.
[c] Dairy experiment farm of Bureau of Dairying, U. S. Department of Agriculture, Beltsville, Md.
[d] Negative.

The quantities of grain refused by the two cows in experiment B were insignificant, except in the case of cow 7 in Period X, she having gone badly off feed for a few days in this period. Some hay was refused by both cows in most of the periods. The quantities of grain and hay consumed in each period are shown in Tables XIV and XV, and these figures show that the cows' appetites were very good almost all through the experiment. The grain offered to cow 54 was reduced somewhat from Period XV on. Her milk yield had become very low when this period was reached, and she went dry altogether in Period XXI. The rather small quantities of hay consumed by both cows in Periods XXIV and XXV must not be taken

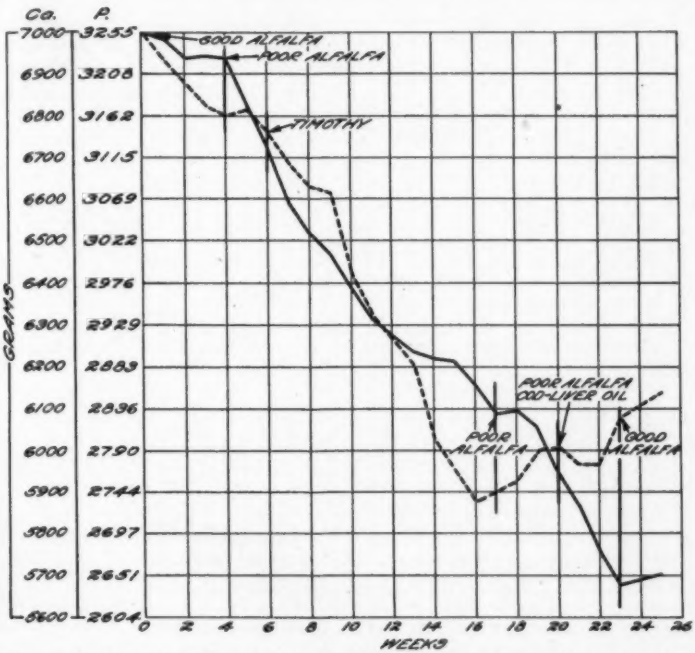

FIG. 1.—Graphs showing the losses and gains of calcium and phosphorus by cow 7 in experiment B. The solid line represents the change in the calcium content in the body during the course of the experiment; the broken line the same for the phosphorus content

as indicating any lack of appetite during those periods. The hay offered was cut down in order to keep the calcium of the ration as low as it had been in the immediately preceding periods.

Experiment B began for both cows on October 6, 1923. On this date they were placed in the experimental stalls, and the feeding of the analyzed rations was begun. Collection of the excreta and milk for analysis was begun on October 8, 1923, and was continued until March 30, 1924.

Figures 1 and 2 give an idea of how calcium and phosphorus were lost from the bodies of the cows throughout the experiment. These figures were made from the results obtained for the calcium and

phosphorus balances. It may be supposed that cows of the size of those used normally contain about 7,000 grams of calcium in their bones (*6, p. 100*). As the ratio between phosphorus and calcium in bone is 1:2.15 (*5, p. 552*), a cow with bones containing 7,000 grams of calcium would have 3,255 grams of phosphorus combined with it.

The left-hand column of figures along the ordinate represents the calcium content of the body. The right-hand column represents the phosphorus content, each figure in this series being obtained from the corresponding calcium figure by dividing by 2.15. The solid line in the chart represents the change in the calcium content of the body during the experiment, and the dotted line the change in the phosphorus content, it being supposed that each cow had 7,000 grams of calcium and 3,255 grams of phosphorus in her body at the beginning. If the animals had lost or gained calcium and phosphorus in just

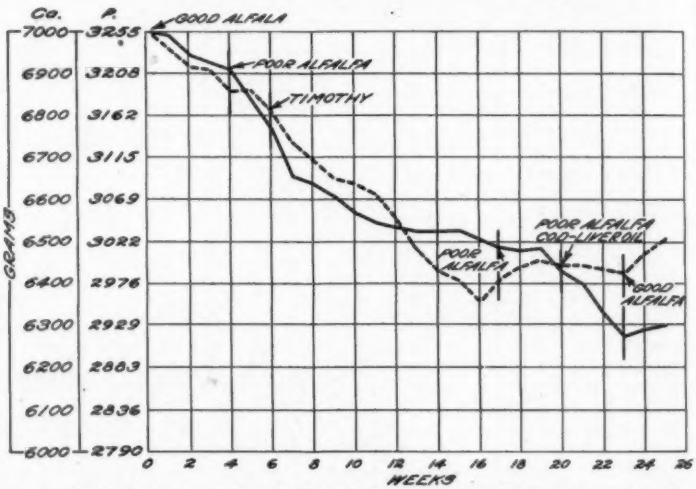

FIG. 2.—Graphs showing the losses and gains of calcium and phosphorus by cow 54 in experiment B. The solid line represents the change in the calcium content in the body during the course of the experiment; the broken line the same for the phosphorus content

the proportion in which they are contained in bone, the two lines would have taken exactly the same course all through the experiment.

In these experiments, however, as in all the others that have preceded them, the losses of calcium and phosphorus do not run exactly parallel. The quantity of phosphorus contained in the body at the end of any given week is sometimes below and sometimes above what it should have been, on the basis of the supposition that only bone material is lost from the body and that the proportion of calcium to phosphorus in the bones remains constant. The soft tissues of the mammalian body contain very little calcium but a good deal phosphorus (*11, p. 165; 12*). It may be supposed, therefore, that phosphorus is being lost from the soft tissues when the phosphorus line is descending faster than the calcium line, as in the first four weeks with both cows. In a period in which the opposite is the case—as in the fifth, sixth, and seventh week with both cows—it may be supposed that phosphorus is being stored by the soft tissues.

It is an important question how far the graphs in Figures 1 and 2 accurately represent the course of calcium and phosphorus metabolism in the experiment. The analyses of the milk and of the mixtures of feces and urine have run very smoothly, and it is felt that errors in these analyses probably have been of relatively small importance. But the discussion of the analyses of the feeds given on pages 844 to 846 indicates that it may be a rather difficult matter to determine accurately the average composition of a large batch of feed, and it seems worth while to consider how far such variations in composition as have been indicated by the analyses might be expected to distort the results.

In Periods XIII, XIV, and XV, for instance, the figures for the balances would indicate that cow 7 lost much more phosphorus than calcium from her body; while in Periods XX, XXI, XXII, and XXIII there was a marked loss of calcium, and this apparently accompanied by a gain in phosphorus. The writers have carefully studied the changes in the batches of feed used in these periods and the magnitude of the errors in sampling or analysis which would be necessary to account for the lack of parallelism between calcium and phosphorus metabolism indicated by the results. The conclusion is that it is difficult to account for more than a small part of the lack of parallelism as due to experimental error, and that the results must be taken to mean that cows may, not infrequently, suffer changes in the phosphorus content of their soft tissues amounting to more than 100 grams in either direction from the average.

The calcium balance showed a fairly consistent relation to the changes in ration in the case of both cows. It was somewhat negative or slightly positive in the first four periods on the good alfalfa hay, it became markedly negative in both cases as soon as the cows were put on poor alfalfa, and it remained negative almost continuously through the subsequent 19 periods on poor alfalfa and timothy hay. In Periods XVIII and XIX, just after the cows were changed from timothy hay back to poor alfalfa, there was for some reason an improvement in the calcium balances, but this was only temporary. In Periods XXI, XXII, and XXIII the calcium losses were practically just as rapid as in Periods V and VI, in spite of the large amounts of calcium that both cows had lost from their bodies in the intervening periods, and in spite of the fact that cod-liver oil was added to the rations of both cows in the three later periods. In Periods XXIV and XXV, when good alfalfa hay was fed again, the calcium balances in both cows became positive.

The results indicate that the calcium of alfalfa hay can be rendered practically unassimilable by allowing the hay to be exposed too long in the field and rained on. The calcium balances were, on the whole, definitely less negative in both cows on the timothy hay than on poor alfalfa, in spite of the much smaller calcium content of the former.

The changes undergone by the phosphorus balances of the two cows show little or no tendency to coincide with the changes made in the rations. Both cows tended to lose phosphorus at a rate which, for the whole experiment, corresponds roughly with the rate of calcium loss. The rations of both cows contained large amounts of assimilable phosphorus all through the experiment, and it seems justifiable to suppose that the phosphorus losses were not caused by

any shortage of assimilable phosphorus in the rations, but were the indirect result of the calcium deficiencies. But the manner in which a loss of calcium from the bones affects the storage or loss of phosphorus by the body seems to be extremely irregular.

Figure 3 shows the manner in which the milk yield of the two cows behaved in the various periods of the experiment. The changes in milk yield were rather irregular, as they always are, but there was, in general, a fairly noticeable tendency for the yield to drop off more rapidly during the periods on poor alfalfa and timothy hay than during those on good alfalfa hay. This is in harmony with other results obtained at the Beltsville station, showing that calcium deficiencies in the diet have a tendency to make the milk yield fall off more rapidly than it otherwise would (*15*). The concentrations of

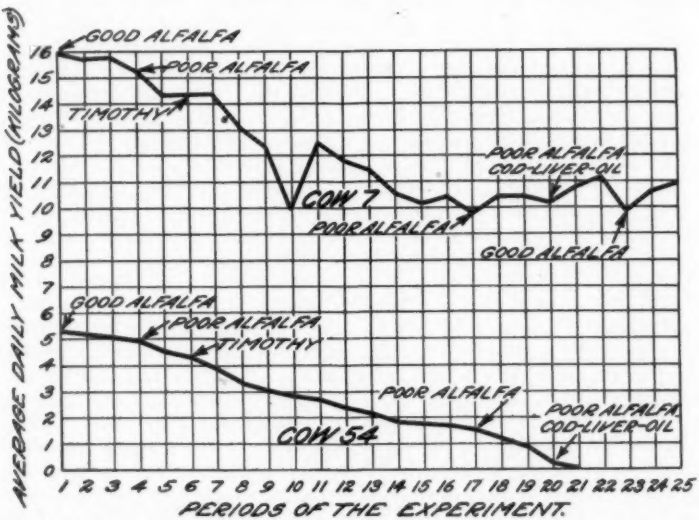
AVERAGE DAILY MILK YIELD (KILOGRAMS)
GOOD ALFALFA
POOR ALFALFA
TIMOTHY
COW 7
POOR ALFALFA
COD-LIVER-OIL
POOR ALFALFA
GOOD ALFALFA
GOOD ALFALFA
POOR ALFALFA
TIMOTHY
COW 54
POOR ALFALFA
POOR ALFALFA
COD-LIVER-OIL
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
PERIODS OF THE EXPERIMENT.

FIG. 3.—Graphs representing the milk yields of the two cows used in experiment B

both calcium and phosphorus in the milk of cow 7 rose noticeably in Periods XXIV and XXV when she was put on good alfalfa hay, while that of the nitrogen did not rise. (See Table XIII.)

The milk yield of cow 7 rose in the first period after she was changed from timothy to poor alfalfa hay, and again during the first two weeks in which cod-liver oil was added to the ration, but it dropped back in the subsequent week, during which the animal was slightly off feed. It rose again quite noticeably during the final two weeks of the experiment on good alfalfa hay, and was higher in this period than during the last three or four weeks of the timothy hay feeding. The normal course of a cow's milk yield after the first few weeks of lactation is always downward, and it is highly probable that the rises which have just been described were connected with the various changes in ration which they accompanied. No similar rises occurred in the case of cow 54, but she had gone dry entirely before the final two periods on good alfalfa hay, and was so nearly

dry even at the beginning of Period XVIII that she can hardly be considered comparable to the other animal.

In Periods XXI, XXII, and XXIII each of the cows received 100 c. c. of cod-liver oil daily in addition to the grain and hay of the ration [4]. This treatment did not produce any consistent improvement, either in the calcium assimilation or in the calcium balance—indeed, on the average, the calcium assimilation was lower in both cows in the oil periods than in the two or three preceding periods on similar rations without the oil. These results are not in harmony with the results of more or less similar experiments with goats at the University of Wisconsin, (*7*) though the improvement in the calcium balances obtained by the administration of cod-liver oil in the Wisconsin experiments was not great.

The cod-liver oil used in this present work was tested for its antirachitic property on rats and was found to have a high potency, according to the firm from which it was obtained. It was also tested for its antirachitic potency on chickens at the University of Wisconsin under the supervision of E. B. Hart, and found to have a high potency for those animals. Several of the results of the present experiments also indicate that it was capable of influencing the metabolism of cows. One of these was the rise in the milk yield of cow 7, and another consisted of certain changes in the composition of the blood which occurred in the case of cow 54.

The rations and history of this animal previous to experiment A had been such as to make it not unlikely that her calcium and phosphorus stores were somewhat depleted when she was put on that experiment. She lost considerable amounts of calcium and phosphorus during experiment A, and it is not likely that she could have made up these losses in the interval which elapsed between the end of that experiment and the beginning of experiment B. Nevertheless, she continued to lose calcium and phosphorus at a fairly rapid rate while on poor alfalfa and timothy hay in experiment B. At the end of Period XVII, the last period on timothy hay in experiment B, blood samples were obtained from both cows. The plasma was separated from the corpuscles and analyzed for calcium, total phosphorus, and inorganic phosphorus. Normal amounts of these elements were found in the plasma of cow 7; but the plasma of cow 54 contained only 8.3 mgm. of calcium, 9.8 mgm. of total phosphorus, and 3.5 mgm. of inorganic phosphorus per 100 c. c. All of these figures are low (*13*) and more or less characteristic of animals suffering from rickets (*17, p. 129, 134–139*).

Another blood sample was obtained from cow 54 at the end of Period XXIII, after she had been on cod-liver oil for three weeks. The plasma calcium had gone up to 9 mgm., the total phosphorus to 11.5 mgm., and the inorganic phosphorus to 5.3 mgm., per 100 c. c. Similar changes in the blood have been brought about in other animals as the result of feeding cod-liver oil (*17, p. 129, 134–139*). The results furnished further evidence that the sample of oil used had antirachitic potency.

Rises in the concentrations of calcium and phosphorus in the blood do not necessarily mean that these elements are being more

[4] Cow 54 received 100 c. c. of the oil daily throughout the three periods. Cow 7 also received 100 c. c. daily, except three days in Period XXIII, when she was slightly off feed and received between 50 and 100 c. c. daily.

rapidly assimilated from the intestinal tract. The bones contain large amounts of them, and their concentration in the blood may be increased by a release of bone material just as well as by an increased rate of assimilation.

The writers think the effects of cod-liver oil on calcium and phosphorus assimilation in ruminants should be investigated further, although the tendency of the oil to throw ruminants off feed, observed in the writers' experiments and also in those at Wisconsin, makes it advisable to be cautious in expecting benefits from its use in practice.

The results just given, taken in connection with the uniformly poor calcium assimilation exhibited by cow 54 throughout experiment B, indicates that when ruminants are fed for long periods on rations such as timothy hay, which are deficient in assimilable calcium, they may get into an abnormal state in which their power of assimilating calcium is lessened in spite of the marked depletion in the calcium stores which has taken place. It is an interesting question how far this condition was aggravated in the case of cow 54 by the long confinement in a building in which she was never exposed to the direct rays of the sun, although there was plenty of diffused daylight.

In Table XVIII the figures for the assimilation of calcium as percentage of the calcium intake have been put together, not only for the writers' present experiments but also for a number of those carried out at the University of Wisconsin along more or less similar lines. Only those cases have been used where the balances were determined while the animals were on the same feed for periods longer than 10 days, and when they had rations with nearly similar calcium and phosphorus contents for several days immediately preceding the periods under consideration

In the table the animals which had the same rations are grouped together, in order to give an idea of the size of the individual variations under the same treatment. The groups are arranged in order according to the average percentage calcium assimilation for each group.

The percentage assimilation of calcium by cows is probably influenced by a number of factors among which perhaps the most important are the general character of the food, the amount of the calcium intake, the milk yield, and the nutritional state in which the animal happens to be at the time the experiment is carried out.

In addition to these, the phosphorus balance and the relation between the quantities of calcium and phosphorus in the diet should be considered. The writers' own results, as shown in Figures 1 and 2, and those of other investigators also, indicate fairly definitely that calcium and phosphorus metabolism are by no means entirely independent of one another (*6, p. 97, 101.*) And there is a considerable body of evidence which indicates that the assimilation of either calcium or phosphorus from the intestinal tract is influenced by the relative amounts of the two elements in the diet (*17, p. 128, 129; 16*).

Table XVIII, therefore, gives not only the percentage assimilation of calcium obtained in the various experiments but also the calcium intake, the milk yield, the relation between the calcium and phosphorus in the diet, the calcium and phosphorus balances, and the general character of the diet.

Perhaps the first conclusion which should be drawn from the figures in Table XVIII is that calcium assimilation is very markedly affected

by the nutritive condition or individuality of the experimental animal. Among the Wisconsin cows which were fed on timothy hay without supplement, for instance, animal 3 assimilated only about 7 per cent of her calcium intake, while animal 2 assimilated more than 20 per cent, and apparently there is nothing in the diet or milk yield of the two animals to explain this difference. Among the Wisconsin cows on lawn grass plus bone meal, again animal 2 had the highest milk yield and the lowest percentage calcium assimilation. Of the two animals used in experiment B at Beltsville, cow 54 has a persistently very poor calcium assimilation. Through the greater part of the experiment she lost more of this element in her urine and feces than she took in with her food.

The results given in Table XVIII point fairly conclusively to the following:

1. The calcium from well-cured or fairly well-cured hays, both timothy and alfalfa, is much better assimilated than is that from bone meal, though the latter, when added to a ration low in calcium, is assimilated to some extent. The average percentage of calcium assimilated from fairly well-cured hay is probably not far from 20.
2. Calcium is decidedly better assimilated from fresh green material than from the same material after it has been dried and made into hay.
3. The manner in which hay is cured makes a great difference in the rate at which calcium is assimilated from it.

In addition to the three statements just made, the writers wish to draw attention to several details of the observations which suggest still other conclusions. These conclusions are perhaps less clearly indicated by the experimental results than are the three above given, but they should be taken into account in the planning of any future experiments.

If one studies Figures 1 and 2 in connection with Table XVIII, one gets many suggestions for the view that calcium and phosphorus assimilation are influenced by one another and by the relative quantities of calcium and phosphorus in the diet. During the first four periods with both cows the phosphorus balances were relatively more negative than the calcium balances [5] (figs. 1 and 2). The proportion of calcium to phosphorus in the rations of both cows was high—2.36 in the case of cow 7 and 1.85 in that of cow 54. In Periods V and VI, in both cases, the proportion of calcium to phosphorus in the rations became less (Table XVIII), and in both cases the phosphorus balances became less negative than they had been before, though the calcium balances became much more negative. In Periods XXIV and XXV with both cows, the proportion of calcium to phosphorus in the rations was still lower than in Periods V and VI; and, in both cases, the phosphorus balances were positive, and relatively more positive than the calcium balances.

The considerations just given suggest that when the rations contain twice as much calcium as phosphorus, or more, milking cows will have difficulty in assimilating sufficient phosphorus to meet their needs. Among the Wisconsin cows given in Table XVIII

[5] In saying that the phosphorus balances were relatively more negative than the calcium balances, it is meant that the lines showing the losses or gains of phosphorus in the figures were descending more rapidly than those showing the losses or gains of calcium—in other words, that the phosphorus losses were greater in proportion to the calcium losses than they would have been if the two elements were being lost in proportions in which they are contained in bone.

there are only three whose rations contained more than twice as much calcium as phosphorus, namely those which received the alfalfa hay cured in windrows. In two of these the phosphorus balances were negative, and relatively more negative than were the calcium balances. All these results indicate that phosphorus assimilation is interfered with by an excess of calcium in the diet, with at least sufficient force to justify further experiments on this question.

Another question on which it seems worth while to comment in the light of the Wisconsin results and the writers' taken together, is that of the curing of hay. The Wisconsin cows assimilated calcium less readily from hay which had been exposed in windrows for four days to the sun, but not rained on, than from hay which was cured under caps with a shorter exposure to the sun. In the case of the cows used by the writers, the comparison was between hay which had been well cured under a shed and hay which had been exposed for several days in the field to both sun and rain. The difference in the calcium assimilation between the better and the poorer hay was much more marked in the writers' experiments than in those at Wisconsin, indicating that much more far-reaching deteriorations are produced in the nutritive qualities of hay by wetting than by fairly long exposure to the sun. In this connection it may be remarked that the nitrogen assimilation by the cows used by the writers was definitely reduced during the periods in which they had the poor alfalfa hay.

SUMMARY OF RESULTS

EXPERIMENT A

The assimilation of calcium by dairy cows is likely to be greatly interfered with by any influence which throws them even slightly off feed.

The results of experiment A indicate that rather small disturbances in a cow's habits and routine are likely to throw her slightly off feed and to interfere very considerably with her calcium assimilation.

When the cows used in this expriment were not disturbed by changes in their routine or by being off feed, they assimilated from 15 to 20 per cent of the calcium contained in a ration of which the calcium content was supplied chiefly by rather inferior alfalfa hay. The percentage assimilation of calcium was not far from the average given by all of the writers' results combined with all of those at the University of Wisconsin, in which the cows had either alfalfa or timothy hay not supplemented with inorganic calcium salts or bone meal.

EXPERIMENT B

The changes in the calcium and phosphorus contents of the bodies of milking cows do not run exactly parallel. A cow may continue to lose one element from her body and gain the other for a period of two or three weeks. Nevertheless, the results show that a long-continued loss of calcium from the body will cause a roughly corresponding loss of phosphorus, even though the ration may contain plenty of assimilable phosphorus.

The rate of calcium loss from the body on deficient rations was slowed up very little by previous losses, within the limits of the experiment. After cow 7 had lost about 15 per cent of her body's store

of calcium she continued to lose this element at about the same rate as in the beginning down to the end of the period during which she was kept on the deficient ration.

The rate at which calcium is assimilated from hay depends very largely on the manner in which the hay is treated after being cut. Results obtained at the University of Wisconsin indicate that calcium is somewhat less well assimilated from hay which has been exposed for four days to the sun than from hay which has been exposed for only a few hours. The writers' own results indicate that calcium is very poorly assimilated from hay which has been rained on. The writers obtained positive calcium balances in a few periods of a week each in a cow that was giving from 10 to 16 kilograms of milk daily, and fed on a ration composed only of grain and well-cured alfalfa hay.

The present results, taken in conjunction with those at the University of Wisconsin, indicate that the average proportion of calcium assimilated by milking cows from fairly well-cured hay is somewhere in the neighborhood of 20 per cent of the intake. About the same percentage of calcium is assimilated from timothy hay with its low calcium content as from alfalfa with its much higher one.

The assimilation of calcium by cows is markedly influenced by peculiarities in their condition which are not yet understood.

In the writers' experiments, the addition of cod-liver oil to the rations had no favorable effect on calcium assimilation. It did seem to have a temporary favorable effect on milk yield.

The milk yield in these experiments showed a general tendency to fall off more rapidly when the rations were deficient in assimilable calcium than at other times.

The writers' results suggest that phosphorus assimilation may be interfered with by an excess of calcium in the ration, and that two parts or more by weight of calcium to one of phosphorus constitute an excess.

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THE PEA APHIS IN CALIFORNIA[1]

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INTRODUCTION

The pea aphis (*Illinoia pisi* Kalt.) has been abundant in many localities on the Pacific coast for a number of years. It has caused considerable damage to leguminous crops, and because of the extensive losses in these crops an investigation of the pest was undertaken in 1915 in California, where the damage had become both severe and an annual occurrence. This paper gives the results of this investigation from a biological standpoint.

ORIGIN AND DISTRIBUTION

According to Davis (*3*),[2] the pea aphis is of exotic origin, having been known in European countries as early as 1815. It has also been reported from South Africa and India. In America it was first observed in 1878, but its destructiveness was not appreciated until 1889. It is now well distributed over the United States and southern Canada, and its range extends even along the west coast of Mexico.

EARLY HISTORY AND DISTRIBUTION IN CALIFORNIA

The first reference to the pea aphis in California was made by Matthew Cooke (*2*) in 1883, who reported it, under the name of *Siphonophora pisi* Kalt., as injurious to peas, but mentioned neither localities nor the extent of damage. The first actual record of injury was made by Chittenden in 1909 (*1*). During that year complaints of injury were received by the Bureau of Entomology of the United States Department of Agriculture as follows: July 31, from San Luis Obispo, on sweet peas; October 3, from Watts, Los Angeles County; October 7, from Santa Ana, Orange County; October 20, from Anaheim; and later from Oxnard, Ventura County.

The horticultural commissioner of Humboldt County, in the northwestern part of the State, also reported the presence of the pea aphis in 1908.[3] Complaints of damage were received by the California Agricultural Experiment Station in May, 1909 (locality not mentioned), and in March, 1910, from Santa Cruz County. These records indicate that the insect had become widely distributed over the State by 1909, but it was not until the seasons of 1910 and 1911 that serious damage was reported. Horticultural commissioners in several southern California counties received reports of severe damage to cover crops of vetch and peas at about this time, and the extensive fields of market and cannery peas in Alameda County were severely damaged for the first time in 1911. Since then the pea aphis has been reported as causing considerable damage in many other counties.

[1] Received for publication Aug. 1, 1925; issued May, 1926.
[2] Reference is made by number (italic) to "Literature cited," p. 881.
[3] Unpublished correspondence.

The pea aphis is distributed practically throughout California (fig. 1). It has been most abundant, and has caused the greatest damage, in the southern and coastal counties, and in the San Francisco Bay region. The map shows a few counties in which it has not

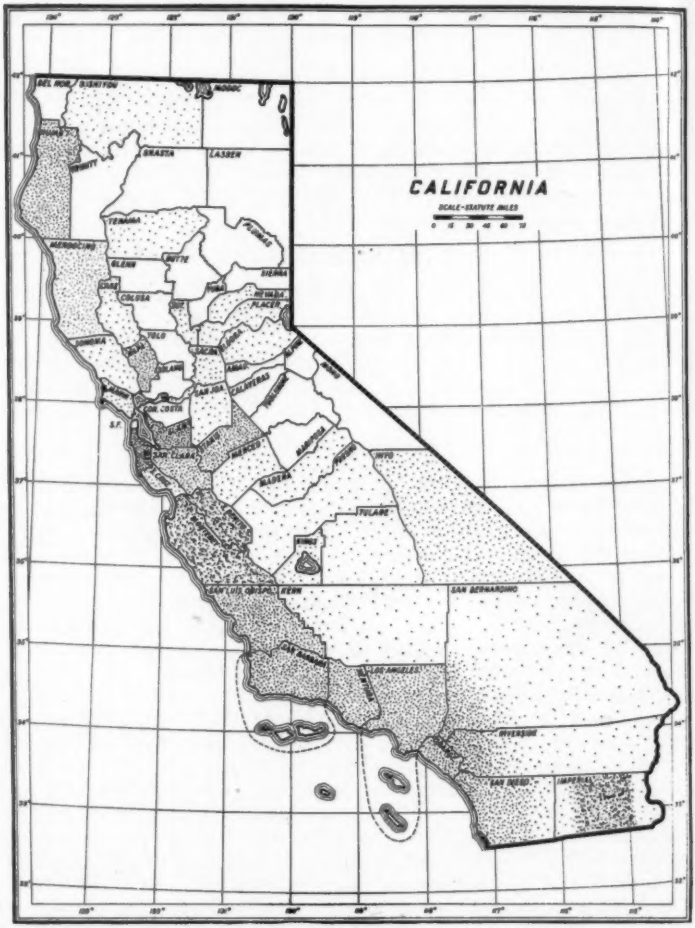

FIG. 1.—Distribution of the pea aphis in California. Heavily shaded counties indicate where it is, or has been, a serious pest, causing considerable damage. Lightly shaded counties indicate where it is a pest of little importance, but largely for the reason that its host plants are not grown to any great extent

been observed, but these are counties in which leguminous crops are not grown commercially, and even if present the insect would attract little attention. It is very probable that a careful search would show that the pea aphis is present to some extent wherever its host plants are grown.

FEEDING HABITS AND CHARACTER OF INJURY

The pea aphis injures the host plant by extracting its juices. This checks the growth, reduces production, and in case of severe attack may often seriously stunt the vines or kill them outright. It not only results in small scrubby plants (fig. 2, A) but, in the case of peas, in a small number of stunted and misshapen pods. In the spring and fall, and on the young vegetation, the aphids prefer the tender leaves and cluster in great numbers about the soft succulent

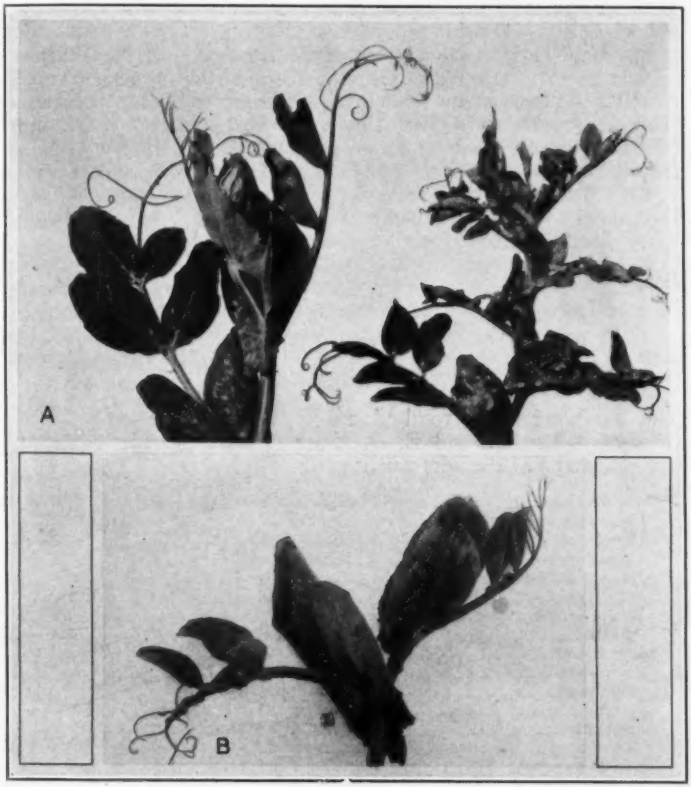

FIG. 2.—A, stunted pea plant (right) showing effect of severe infestation of the pea aphis compared with an unaffected plant; B, a colony of the pea aphis getting a good start in the terminal cluster

stems and terminal buds (fig. 2, B). During the hotter or dryer weather of summer and the colder weather of winter and on older plants, the aphids are likely to be scattered and many are found on the undersides of the lower leaves.

EXTENT OF INJURY

In former years common vetch and field peas were used extensively for cover crops in the orchards of southern California. Largely

because of the ravages of the pea aphis, however, both were finally abandoned in favor of sweet clover (*Melilotus indica* (L.) All.), on which the aphis does not thrive.

In San Mateo County there were formerly large acreages of market peas, but these also have largely been abandoned.

Recently a considerable acreage of purple vetch has been grown as a cover crop, but in 1923, and particularly in 1924, the infestation of the pea aphis was so severe that in many orchards the vetch was killed.

Seed companies have had considerable difficulty for a number of years in growing flowering sweet peas for seed on account of the damage caused by the pea aphis and have practically given up the attempt, except in certain cool localities adjoining the coast, and even in these places it has been necessary to practice control measures at times. In Alameda County, in several different years, market and cannery peas have been so severely injured by the pea aphis as to reduce the total crop 30 to 50 per cent. The greatest damage occurred in 1921, when the crop of market peas was reduced more than 50 per cent and the pack of canned peas 70 per cent. In 1922 the first crop of alfalfa in Inyo County was checked by an infestation, and in 1923 the first crop in most of the alfalfa-growing sections of southern California was severely damaged by the worst infestation known to have occurred on alfalfa in the State up to that time. An even more severe, though local, infestation occurred in the Antelope Valley in 1925.

FOOD PLANTS[4]

The following list of food plants in California is based on observations of the writer and E. O. Essig (*4*), W. M. Davidson, A. F. Swain (*9*), and V. L. Wildermuth:

Garden peas	*Pisum sativum* L.
Canadian field peas	*P. arvense* L.
Purple vetch	*Vicia atropurpurea* Desf.
Common vetch	*V. sativa* L.
Narrow-leaf vetch	*V. angustifolia* Nees.
Wild vetch	*V. americana* var. *angustifolia* Nees.
Broad beans	*V. faba* L.
Alfalfa	*Medicago sativa* L.
Bur clover	*M. hispida* Gaertn.
Sweet peas	*Lathyrus odoratus* L.
Wild peas	*Lathyrus* sp.
Lupine	*Lupinus* sp.
Fenugreek	*Trigonella foenum-graecum* L.
Beans	*Phaseolus* sp.
Leather root	*Psoralea macrostachya* DC.
Pansy	*Viola* sp.
Nettle	*Urtica holosericea* Nutt.

Of these plants the pea aphis causes the most damages to garden, field, and sweet peas; vetches grown for food or seed; vetch and fenugreek grown as cover crops; and, more recently, to alfalfa.

Other plants have been recorded as hosts of the pea aphis, but these are for the most part incidental, becoming temporarily infested during a migration. Many experiments have demonstrated that breeding does not continue on these incidental hosts.

[4] Determination of most of the commoner host plants was made by the writer, and the others by Roland McKee and Leroy Abrams.

DISPERSION

Dispersion of the pea aphis is accomplished by both the winged and wingless forms, the former migrating from field to field, and the latter moving from plant to plant. At certain times of the year, owing to factors not entirely understood, large numbers of winged forms are produced. Occasionally a migration will be observed when the air seems full of flying forms. In colder climates these migrations of winged forms are periodic, the aphids regularly moving from one food plant to another at certain seasons of the year. In the milder climate of the Pacific coast the migrations are not ordinarily so noticeable or regular, as the growing seasons are longer and hence there is no necessity for migration to new food plants. In the San Joaquin Valley, where there is a large acreage of alfalfa, a migration of aphids which have been developing on these plants for several months commonly occurs in April. In 1923 this movement was very extensive, and continued for two weeks. It was heaviest during the warmest part of warm days, that is, at temperatures of 80° to 90° F. in the shade. A severe infestation of all pea fields resulted, although some of them were as far as 5 miles from the nearest alfalfa. Smith (*8*), in Virginia, reported a migration of winged individuals to their summer food plants, which began on June 22. By July 1 winged females could be found at various distances up to 1½ miles away. The greatest distance was gained by individuals going with the wind. At the same time wingless individuals were observed crawling over fallow ground 30 or 40 feet from infested pea vines.

With many species of aphids an infestation may be more or less localized and the damage may occur in spots, but with the pea aphis the infestation usually is distributed over the entire field. However, in heavy infestations injury may be more severe in spots, resulting in many areas of dead plants scattered over the field.

LIFE HISTORY AND HABITS[5]

The life history of the pea aphis in the mild climate of California is exceedingly simple, being a continuous series of generations of viviparous females except in some of the inland or mountain regions where under the influence of cold weather the winter may be passed in the egg stage. Reproduction continues throughout the year, and is retarded only by the colder weather of the winter and by lack of food at other times. The warm weather of summer, together with a lack of abundance of food plants, greatly reduces the total number of aphids and the length of life of individuals, but the rise in temperature increases the rate of growth and reproduction. In fact, as will be shown later, there is a direct relation between the temperature and the rate of growth and reproduction.

In all the different series of generation experiments, in which several hundred individuals were reared, in the numerous stock cages and in all the field observations made during this investigation no amphigenous forms or eggs were found until November 6, 1925, when a search of the alfalfa fields in the Antelope Valley, where a heavy infestation occurred in the spring, resulted in finding eggs. This section is in the

[5] For complete description and synonymy, see Davis (*3*).

southeastern part of the Mojave Desert, where winter temperatures approach zero, and where a minimum of 20° F. had already been reached in 1925.

SEASONAL HISTORY

Although the pea aphis can be found during any month of the year in California, it is most abundant in the spring and fall. Active viviparous females occur throughout the winter. With the advent of warmer weather reproduction is greatly accelerated, which usually results in severe infestations during the latter part of March or early in April. These infestations continue until the rains are over and the weather becomes dry and warm. About this time, also, the plants are reaching maturity and becoming less succulent. These conditions, occurring usually during June in California, cause a marked decrease in the number of aphids, so that beginning with July they become scarce. About the middle of September or the first of October the numbers again increase, and the fall crops of peas are often seriously infested during October and November. With cooler weather and retarded reproduction, the numbers decrease. Occasionally market peas are injured in December or January, and in 1924 the most serious infestation of alfalfa known in the State occurred in February. The serious infestations have all been during periods when the mean temperature was above normal.

GENERATION EXPERIMENTS[6]

Several series of generation experiments were conducted with the pea aphis at Hayward, in central California, and at Alhambra and Pasadena, in southern California. In all experiments the rearing methods were adapted from Phillips and Davis (*6*). Individual aphids were observed on potted pea plants, the latter inclosed in lantern chimneys with screen covers over the tops. These cages were kept in a screened outdoor insectary.

In the first series a winged viviparous adult was taken in the field at Hayward, Calif., March 8, 1915. This series was discontinued on December 15, 1915, by which date a maximum of 15 generations had been obtained. The second series, begun January 1, 1916, from a wingless viviparous adult taken in the field, was discontinued June 30, when the station at Hayward was abandoned. The tenth generation had been reached in the six months.

The third series was begun at Pasadena[7] January 1, 1917, from a wingless viviparous adult taken in a pea field, and continued at Alhambra to July 31, 1918, when the experiment was concluded owing to the death of the food plants. During these 19 months a maximum of 44 generations was produced. The minimum, or last-young series, reached the fourteenth generation on November 11, 1917, at which time the maximum, or first-young series, had reached the twenty-sixth generation. The last aphid of the last young series reached maturity, and its abdomen became distended with young, but none was born. The abdomen turned a greenish brown, and the aphid died a month later. Eighteen young were dissected from the abdomen, all dead, and some had turned brown.

[6] Victor Duran conducted most of the generation experiments at Alhambra, and also assisted in many of the field observations and experiments.

[7] Hereafter this will be referred to as the Alhambra series.

MOLTS

The pea aphis passes through five instars, molting four times. Both Davis (*3*) and Smith (*8*) reported four molts. Folsom (*5*), on the other hand, observed one instance of five molts, and in the 1915 series of life-history experiments conducted by the writer at Hayward five molts were observed in several instances. In each case it required the usual length of time between each of the five molts, and between the last molt and the production of the first young. The instances occurred in several different generations of the series which started from a wingless female. The molted skins were not retained, so the accuracy of these observations may be open to question.

LENGTH OF INSTARS

The instars, exclusive of the adult stage, extend from 1 to 8 days, the average for the year being 3 days. The instars are noticeably longer during the colder months. Table I gives the average length of each instar for the different seasons of the year at Hayward, Calif.

TABLE I.—*Average length of each instar of the pea aphis, in days, for the four seasons, in the generation series conducted in central California in 1915 and 1916*

Number of individuals	Season	Mean temperature	First instar	Second instar	Third instar	Fourth instar	Age on reaching maturity
		°F.	Days	Days	Days	Days	Days
6	January, February	52.0	3.8	3.8	3.8	4.6	16.0
41	March, April, May	59.2	2.8	2.6	3.0	3.2	11.6
24	June, July, August	------	2.1	2.2	2.0	2.3	8.6
9	September, October, November	------	3.0	2.8	3.2	4.2	13.2
80	Yearly average	------	2.9	2.9	3.0	3.6	12.4

In southern California, under the influence of a warmer climate, the life history is shortened somewhat, as is shown in Table II.

TABLE II.—*Average length of each instar of the pea aphis, in days, for the four seasons, in the generation series conducted in southern California in 1917 and 1918*

Number of individuals	Season	Mean temperature	First instar	Second instar	Third instar	Fourth instar	Age on reaching maturity
		°F.	Days	Days	Days	Days	Days
34	December, January, February	52.5	3.5	3.6	3.8	4.7	15.6
30	March, April, May	57.5	3.2	2.7	3.1	3.6	*12.6
63	June, July, August	71.9	1.7	1.8	1.8	1.8	7.1
39	September, October, November	66.6	1.9	2.1	2.2	2.5	8.7
166	Yearly average	61.9	2.6	2.6	2.7	3.2	11.0

* Mean temperature at Hayward for March, April, and May in 1916 was a little higher than at Pasadena in 1917, which caused a shorter period of immaturity in the former locality for these three months.

For comparison, Table III gives the length of the instars as reported by Smith (*8*) in Virginia.

TABLE III.—*Average length of each instar in days for the seasons during which the pea aphis is active in Virginia as compiled from Smith (8, p. 62 and Table I)*

Season	Mean temperature	First instar	Second instar	Third instar	Fourth instar	Age on reaching maturity
	° F.	Days	Days	Days	Days	Days
Spring	62.8	1.2	3.2	3.5	3.4	11.3
Summer	70.9	1.2	2.2	2.3	2.2	7.9
Fall	63.5	2.4	2.8	3.0	3.0	11.3
Average for season	65.7	1.6	2.7	2.9	2.9	10.2

AGE AT WHICH REPRODUCTION BEGINS

The production of young begins very soon after maturity is reached, the time being influenced by temperature. At Hayward it began from 1 to 6 days after maturity, averaging 2.8 days for the year. The age at which reproduction began was from 9 to 23 days, averaging 15.2 days for the entire series.

At Alhambra during the summer many of the adults produced their first young the same day as that on which their last molt occurred, whereas in winter the time from maturity to the birth of the first young ran as high as 6 days. The average for the year was 2 days. Here the age of the aphids when the first young were produced varied from 8 to 32 days, averaging 12.7 days.

In Indiana, Davis (*3*) found that the females gave birth to the first young at ages ranging from 6 to 35 days according to the time of year, with an average of 12.1 days during the season from May to December. In Virginia, Smith (*8*) found that the age was from 7 to 26 days, and averaged 12.5 days.

LENGTH OF THE REPRODUCTIVE PERIOD

The active reproductive period—that is, the number of days from the birth of the first young to the birth of the last young—is also influenced by weather conditions, varying inversely with the temperature.

At Hayward, Calif., the length of the reproductive period ranged from 2 to 32 days, averaging 17.3 days. At Alhambra the same period ranged from 1 to 43 days, averaging 14.6 days. The minimum and average are lower at the latter place on account of the fact that occasionally there is a hot, dry spell of several days' duration which kills large numbers of the aphids.

Weather conditions usually determined the length of adult life after the production of the last young. Where such conditions were favorable the adult often survived for several days, but when conditions became unfavorable it usually died either on the date of birth of the last young or the day following. Hot weather was unfavorable; cool weather favorable. Aphids which produced a large number of young survived longer after the birth of the last young than those which produced few.

In Virginia (*8, p. 63*) the length of the reproductive period was found to range from 4 to 36 days, with an average of 18.1 days; in Indiana (*3*) it ranged from 2 to 68 days, with an average of 22 days.

At Hayward, Calif., the length of time between the birth of the last young and the death of the female was from 0 to 16 days, averaging 2.5 days: at Alhambra it was from 0 to 14, averaging 1.8.

LONGEVITY

Temperature exerts a marked influence on the longevity of the pea aphis. As the temperature rises the length of life becomes shorter, and, conversely, as the temperature goes down the length of life increases. Figure 3 shows that all periods in the insect's life, the instars, length of time between maturity and birth of first young, the reproductive period, and the time between the birth of the last

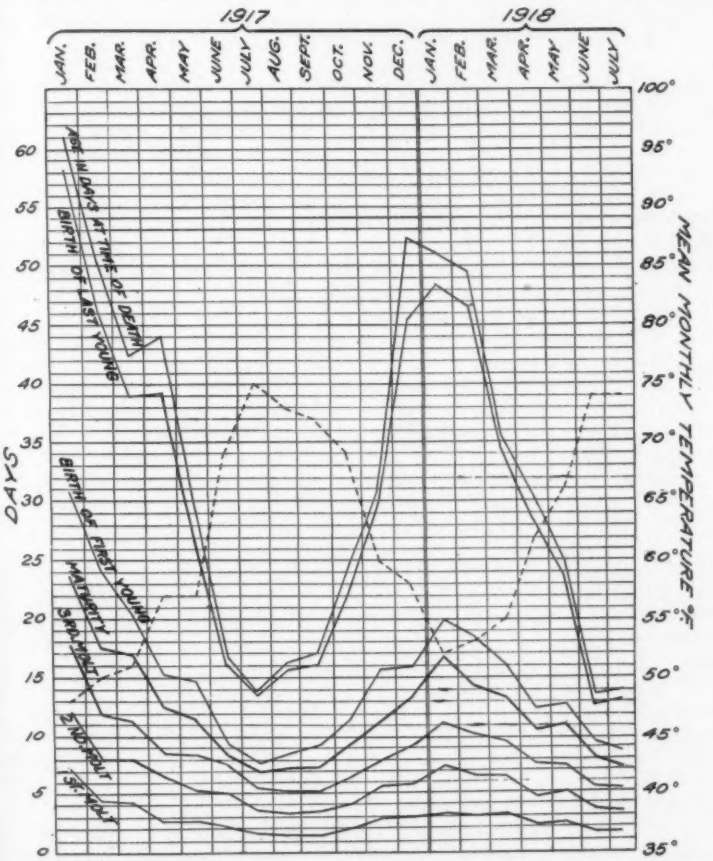

FIG. 3.—Graph showing the effect of the average monthly temperature on the length of the different periods in the life of *Illinoia pisi* (for the series begun in January, 1917, and continued into July, 1918). The broken line shows the average monthly mean temperature at Alhambra, Calif., and the figures at the right of the chart showing °F. are to be read only in connection with this line

young and death, are all affected by temperature, the periods shortening as the temperature rises in the spring, and lengthening as it declines in the fall.

At Hayward, Calif., the entire length of life from birth to death was found to range from 11 to 55 days, averaging 31.7 days; at Alhambra the minimum length of life was 10 days and the maximum

77, with an average for more than 200 individuals throughout the year of 26.6 days. The lower average at Alhambra was brought about by increased mortality during the hotter summer weather in southern California.

Davis (*3*) reported the length of life in Indiana to be from 10 to 85 days, averaging 39.1 days. In Illinois, Webster (reported by Folsom (*5*)) found this period to range from 13 to 50 days, with an average of 25.4 days. In Virginia, Smith (*8*) reported a minimum of 12 and a maximum of 56 days, with the average length of life (excluding hibernating individuals) 31.7 days.

FECUNDITY

The pea aphis is a prolific species, having not only a long reproductive period, but also producing a considerable number of young per day. At Hayward, in 1915, the number produced per day ran as high as 13, and averaged 4.08. In 1916 the highest number of young produced in one day was 14, with a seasonal average of 4.13. At Alhambra, the maximum number produced in a day was 14, and the average was 4.1. These average figures are for the active reproductive period only and do not take into consideration the few days between maturity and the production of the first young, or the time between the last young and death.

It was observed that in most cases the average number of young produced in a day did not vary greatly, except toward the end of the reproductive period in the case of the longer-lived aphids. In warm weather, with the exception of the last few days (almost the last half when the reproductive period is longer than 20 days), the average number produced in a day was between 4 and 5. Thus, if the aphid had a reproductive period of 10 days the total number of young would be about 40 to 50, and if it had a reproductive period of 20 days the total number would be about 80 to 100. A reproductive period longer than 20 days almost invariably resulted in the production of more than 100 young. The highest number produced in one day by the average female was reached from the fifth to the seventh day of the reproductive period. The number then remained fairly constant for about eight days, when it gradually declined, except for those having a reproductive period of 10 days or less.

Figure 4 presents graphically the average number of young produced per day, in four groups of 10 or more aphids, each group having reproductive periods of different lengths. This figure shows that the shorter the reproductive period the higher the number produced per day, and also that the shorter periods end much more abruptly than the longer ones.

It will be noted that for the aphids having a 10-day reproductive period, the average number per day began a little above 5, ran up to more than 7 on the fifth day, and then declined to 4 on the tenth or last day. With a 20-day reproductive period, the number produced per day began above 5, ran up to 6½ on the seventh day, remained above 4 to the fifteenth day, and then declined to 2½ on the twenieth or last day. For those with a 29-day reproductive period, thet number per day began below 4, rose to a little above 6 on the seventh day, remained above 4 to the sixteenth day, and then declined to 1 on the last day. For the longest period, that of 35 days, the number per day began a little above 3, rose to 6½ on the seventh day,

dropped to above 3 on the ninth day, then rose and remained above 4 until the fifteenth day, declining again on the sixteenth to above 2 and rising from the sixteenth to the twenty-first day to above 4; varying from the twenty-third to the twenth-eighth day, and remaining less than 1 for the last 8 days.

Thus, it is seen that although the difference is not very great the longer-lived aphids average fewer young per day than the shorter-lived ones, and that with the former the number per day remains high to the last day, whereas with the latter the number declines considerably toward the end of the reproductive period. It is also apparent that although the average per day is fewer for the longer-lived aphids the difference is so small that the average total number of young increases almost in direct proportion to the length of the reproductive period.

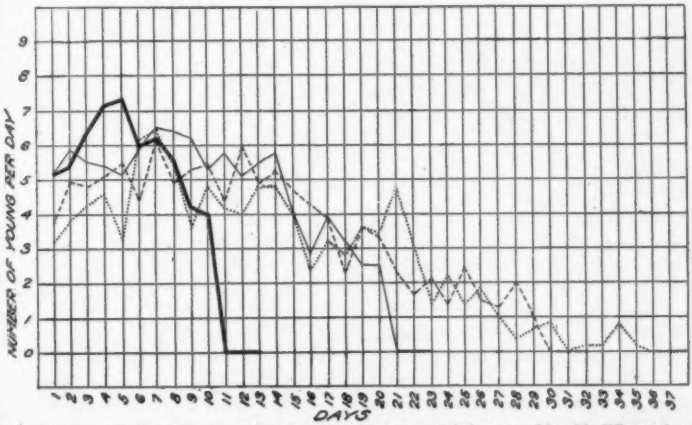

FIG. 4.—Average number of young produced per day for 4 groups of the pea aphis with different lengths of reproductive periods. The heavy line indicates a reproductive period of 10 days, the light line 20 days, dashes 29 days, dots 35 days (Alhambra, Calif.)

In studying the reproduction of the pea aphis the insects were divided into four groups having different lengths of reproductive periods. The total production of young for these four groups is shown in Table IV.

TABLE IV.—*Total production of young for four groups of the pea aphis having reproductive periods of different lengths*

Length of reproductive period	Maximum number of young	Minimum number of young	Average number of young	Average number of young per day
Days				
10	76	42	57.4	5.74
20	111	73	98.5	4.93
29	136	74	107.5	3.70
35	176	70	114.4	3.27

An examination of the data at hand shows that the 10-day periods occurred in the hotter part of the summer; the 35-day periods in the cooler part of the winter; and the intermediate lengths occurred over a longer period between the two extremes.

Further to show that high or low fecundity and short or long life are influenced by environment, and are not hereditary, a study of the parent and offspring of some of the individual aphids from Figure 4 was made. The results are given in Table V. These indicate that such factors as fecundity and length of life are not transmitted.

TABLE V.—*The number of young and the length of the reproductive period for certain aphids from Figure 4, together with the same data for the parents of these aphids and the offspring*

Parent		Individual aphids from Figure 4		Offspring	
Total number of young	Length of reproductive period	Total number of young	Length of reproductive period	Total number of young	Length of reproductive period
	Days		*Days*		*Days*
108	27	45	10	91	24
11	3	45	10	73	10
98	14	62	10	24	5
16	4	44	10	58 5	[a] 8 4
41	6	68	10	77	13
127	31	88	20	127	31
45	10	90	20	76	11
47	8	101	20	36 99	10 15
108	31	102	20	110	30
106	30	76	29	135	28
91	30	108	29	102	21
107	24	120	29	127	31
89	29	136	29	90	13
52	39	70	35	68	[a] 18
77	39	76	35	81	25
110	18	176	35	115 118	32 30

[a] Record incomplete; aphid escaped.

At Hayward, in 1915, the total number of aphids produced by individual females, omitting any which died before producing young, ranged from a minimum of 5 to a maximum of 121, with an average of 65.9. In 1916 the minimum was 3 and the maximum was 176, with an average of 67.5 young per female. At Alhambra the number ranged from 3 to 136, averaging 68.8. Although an average of from 65 to 68 is high, it must be borne in mind that in the favorable weather occurring during the spring and fall infestations the number produced is much higher, often running more than 100. In the series conducted at Hayward in 1915 (14 aphids) and 1916 (8 aphids) each aphid produced more than 100, and at Alhambra each of 45 aphids produced more than 100, these 45 representing almost 25 per cent of the total number used.

In Virginia the females produced 1 to 11 young a day, averaging 4.8; the maximum number produced by a single female was 142, and the average 80.7. In Indiana the largest number produced a day was 14, and the daily average was 3.7; the maximum total number produced by an individual female was 124, and the average was 68.3. In Illinois the maximum number was 147, and the average 46.

COMPARISON OF WINGED AND WINGLESS FEMALES

It was observed early in the experiments that winged females not only developed more slowly but also produced fewer young than did the wingless forms. Table VI gives a comparison of the instars,

length of life, and number of young of the two forms, taken from records of 27 winged and 20 wingless individuals in the 1915 series at Hayward.

TABLE VI.—*Comparison of life history and number of young produced by 27 winged and 20 wingless forms of Illinoia pisi, at Hayward, Calif., in 1915*

	Form	
	Wingless	Winged
	Days	*Days*
First instar	2.64	2.74
Second instar	2.50	2.59
Third instar	2.40	2.81
Fourth instar	2.65	3.29
Length of immature period	10.19	11.43
Period between last molt and birth of first young	1.90	3.50
Age at birth of first young	12.09	14.93
Length of reproductive period	15.80	16.40
Total length of life	29.60	32.90

Average number of young: Wingless, 71.70; winged 58.30.
Average number of young per day during reproductive period: Wingless, 4.54; winged, 3.55.

From Table VI it is apparent that one more day is required for the winged form to develop than for the wingless, and that most of this excess time is taken up in the fourth instar, during which the greatest development of the wing pads takes place. The winged forms are also a day or more later in beginning to produce young, and although the length of the reproductive period is practically the same the winged form averages about one young per day less than the wingless forms.

In Virginia, Smith (*8*) compared the development of six winged and six wingless females, and obtained results similar to those secured by the present writer (Table VII). Smith's experiments were made during October and November, when the development was much slower than it would have been in the summer or early fall.

TABLE VII.—*Comparison of the development and number of young produced by wingless and winged females in Virginia,[a] as compiled from Smith (8, p. 61)*

	Form	
	Wingless	Winged
	Days	*Days*
Age at birth of first young	17.6	23.3
Length of reproductive period	15.5	14.8
Total length of life	40.6	48.8

Average number of young: Wingless, 57.7; winged, 45.1.
Average number of young per day during reproductive period: Wingless, 3.8; winged, 3.0.
[a] Aphids kept in greenhouse having a mean temperature during the period of the experiment of 60° minimum and 80° F. maximum.

NUMBER OF GENERATIONS A YEAR

The only series carried on for more than a year was reared at Alhambra. From an individual born January 1, 1917, a maximum of 28 generations was reached by December 31 of that year, whereas the minimum number for the same period was 14. The maximum

was obtained by taking the first-born in each generation and the minimum by taking the last-born of the last individual in each generation.

At Hayward, from March 8 to December 15, 1915, a period of nine months, 15 generations were produced, and from January 1 to June 30, 1916, the tenth generation was reached. These two figures, however, do not represent the maximum number of generations possible, for in a number of cases the first-born were not used to continue the series. These figures more nearly approach an average than a maximum. In Virginia, from March 19 to December 5 a maximum of 22 generations was produced. In Indiana, 19 generations were produced between March 31 and the following January. In Illinois the seventeenth generation was reached between March 23 and September 21 (Table VIII).

TABLE VIII.—*Comparison of life-history data of the pea aphis at Hayward and Alhambra, Calif., and Virginia, Indiana, and Illinois, as compiled from Smith (8) and Davis (3)*

Locality	Age when reproduction begins			Length of reproductive period			Total length of life		
	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average
	Days	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*
Hayward	23	9	15.2	32	2	17.3	55	11	31.7
Alhambra	32	8	12.7	43	1	14.6	77	10	26.6
Virginia	[a] 26	7	12.5	36	4	18.1	[a] 56	12	31.7
Indiana	35	6	12.1	68	2	22.0	85	10	39.1
Illinois						12.1	50	13	25.4

Locality	Number of young per day			Total number of young			Length of seasons	Number of generations per season (or year)	
	Maximum	Minimum	Average	Maximum	Minimum	Average		Maximum	Minimum
							Months		
Hayward	14		4.08	176	3	67.5	[b] 9¼	15	
Alhambra	14		4.01	136	3	68.8	12	28	14
Virginia	11	1	4.80	142		80.7	8½	22	
Indiana	14		3.70	124		68.3	9	19	12
Illinois	13			147		46	6	17	

[a] Three hibernating females averaged 118 days from birth to production of first young, and 156 days for the total length of life.

[b] Life-history studies were carried on for only 9¼ months although at Hayward the pea aphis is active throughout the year.

NUMBER OF GENERATIONS PRODUCED PARTHENOGENETICALLY

In the life-history experiments conducted, representing close observation of more than 350 aphids in individual cages as well as numerous observations in stock cages and in the fields, no males or oviparous females were found. All generation experiments, except one series, were discontinued, but the latter, begun January 1, 1917, continued until August 14, 1918, a period of 19½ months, when the last individual in the series died, being then in the forty-fourth generation. Since series 3 continued through most of two years, it is possible to compare the activities of the insects in the second year with earlier generations in the first year. Figure 5, A, gives the aver-

age number of young produced in a day for each month from January to July, inclusive, for 1917 and 1918. During the entire seven months, with the exception of July, the mean temperature was from 4° to 9° lower in 1917 than in 1918, and the number of young produced daily was correspondingly less.

Figure 5, B, shows the monthly averages of the total number of young per aphid. For the first three months of the second year the number of young per aphid averages more in 1918 than in 1917, but for the remaining four months the 1918 average is less. This may have been occasioned by the fact that when the series was

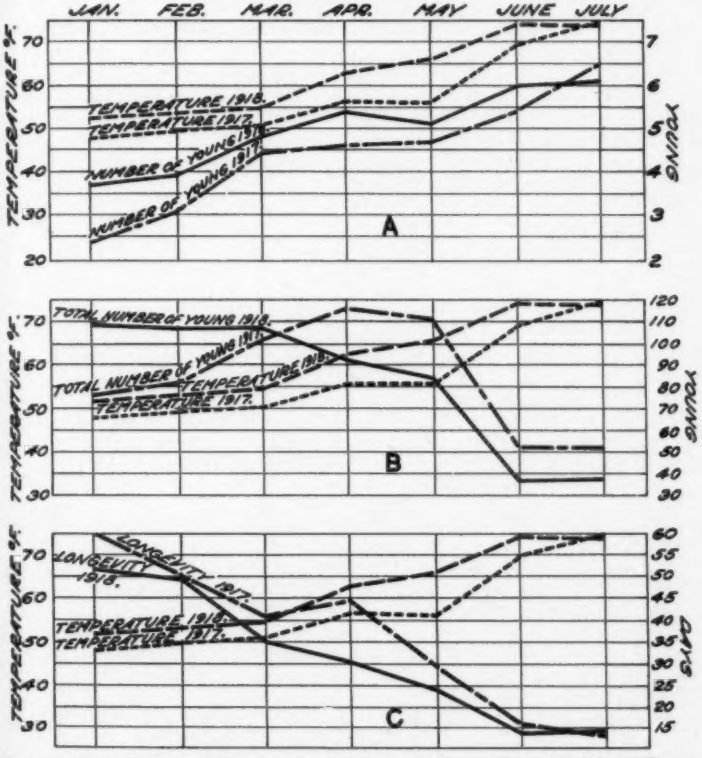

FIG. 5.—A, average daily production of young for the corresponding months of 1917 and 1918; B, average total number of young per aphid for the corresponding months of 1917 and 1918; C, average length of life of the aphid for the corresponding months of 1917 and 1918

begun in 1917 there were fewer individuals to average than in the corresponding months of 1918.

Figure 5, C, shows the monthly averages of the total length of life. Here the curves follow each other in much the same fashion as do the mean temperature curves.

These graphs seem to support the contention that the series ended in the forty-fourth generation on account of adverse weather and food conditions rather than on account of long continuous parthenogenetic reproduction. It is probable that with weather and food conditions favorable this parthenogenetic reproduction

would have continued indefinitely. Davis (*3*) observed that in Indiana sexual forms which produce the overwintering eggs are common; in the latitude of Tennessee they are rare, the winter being ordinarily passed as viviparous females; and he believes that still farther south, where only viviparous females have been observed, the species may continue to produce viviparously for an indefinite period. Russell (*7*) reports as follows in regard to a closely related species, *Macrosiphum rosae:*

> In a climate as mild as that of southern California this insect reproduces continuously throughout the year and undoubtedly is capable of reproducing a sexually and viviparously for an extended period.

EFFECT OF TEMPERATURE ON RATE OF GROWTH AND REPRODUCTION

Temperature has a marked effect on the activities of the pea aphis. Figure 3 shows that the rate of growth and length of life vary inversely with rise or fall in the temperature. A rise or fall of 10° in the mean temperature will shorten or lengthen the life of the aphid by 15 to 20 days. The mean winter temperature of 48° to 55° F. gives a total length of life of 35 to 60 days, whereas the summer mean temperature of 70° to 75° results in shortening the life to 13 to 18 days.

All of the different periods in the life of the pea aphis are correspondingly shortened or lengthened by a rise or fall in the temperature. Beyond a certain point not definitely established high temperatures are harmful. It has often been observed that a period of hot weather, especially when accompanied by low humidity, is very effective in checking an infestation of this aphis. The length of life and the total number of young produced are not only greatly reduced, but a great many of the young aphids die before reaching maturity.

The effect of temperature on the number of young produced per day is just the opposite of that on the length of life, the latter varying inversely and the former directly with the temperature. This is shown in Figure 6. The number of young produced per day increases or decreases as the temperature rises or falls. The chart shows that, with the exception of one month, the curve indicating the average number of young produced in a day follows the temperature curves.

In spite of the fact that more young are produced per day in warm weather, the total number of young per aphid is much less then than in cool or cold weather, because the reproductive period is greatly shortened by the higher temperature.

NATURAL CONTROL

WEATHER

Mention has already been made of the effect of temperature on the pea aphis. Often infestations have been wiped out by hot weather and sometimes they have been greatly retarded by cold weather. In southern California hot, dry winds from the desert sometimes occur during the fall. At such times the humidity is very low and the atmosphere is in a condition commonly called "electrical." Such weather conditions are destructive to the pea

aphis and usually materially check an infestation. Heavy rains dislodge and kill many aphids, particularly those exposed in the terminal clusters.

PREDACIOUS ENEMIES

SYRPHIDAE

The most important predacious enemies of the pea aphis in California are the syrphid flies, the larvae of which feed voraciously on any stage of their host. These larvae are all somewhat similar in shape, but some appear smooth and sluglike, and others are rough and spiny (fig. 7, A and B). They move about blindly over the plant, waving the anterior part of the body until it comes in contact with an aphid, which is seized and the body juices sucked out. The larvae of the larger species of Syrphidae, such as *Scaeva pyrastri* L., are each capable of destroying more than 500 aphids, while a larva of the smaller species, such as Allograpta, may destroy as many as 200 to

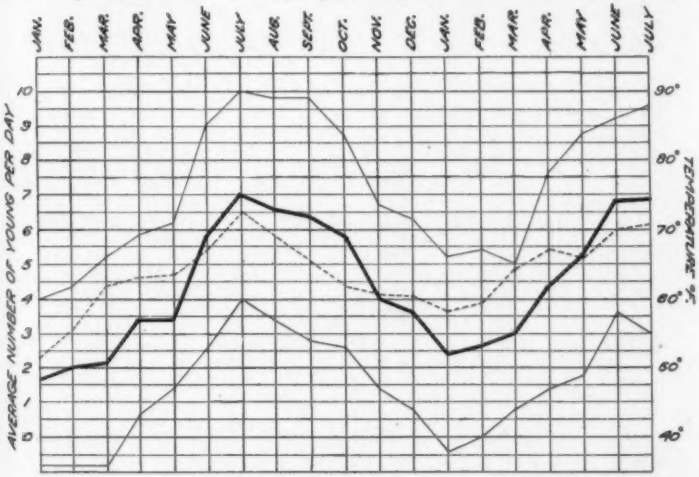

FIG. 6.—Graph showing the effect of temperature on the average number of young per day for each month of the year at Alhambra, Calif. The heavy line shows the average monthly mean temperature, the upper and lower lines the average monthly maximum and minimum temperatures, and the broken line the average number of young per day

300. The following is a list of the common species which feed on the pea aphis, given in the order of their importance: *Scaeva pyrastri* L., *Eupeodes volucris* O. S., *Syrphus opinator* O. S., *Syrphus nitens* Zett., and *Allograpta obliqua* Say. In addition, the following have also been taken, though more rarely: *Allograpta fracta* O. S., *Sphaerophoria sulphuripes* Thom., *Syrphus torvus* O. S., *Melanostoma obscurum* Say, *M. trichopus* Thom., and *M. chaetopoda* Davidson.

The general life history of these predators is as follows: Egg stage 3 to 9 days; larval stage 12 to 21 days; pupal stage 8 to 18 days; a total length of life from egg to adult of approximately 3 to 7 weeks. Females are capable of producing as many as 175 eggs during their life of about three weeks.

Owing to the fact that the aphids multiply more rapidly than the syrphids, an infestation often becomes serious in spite of the presence of these natural enemies. However, they undoubtedly assist in either

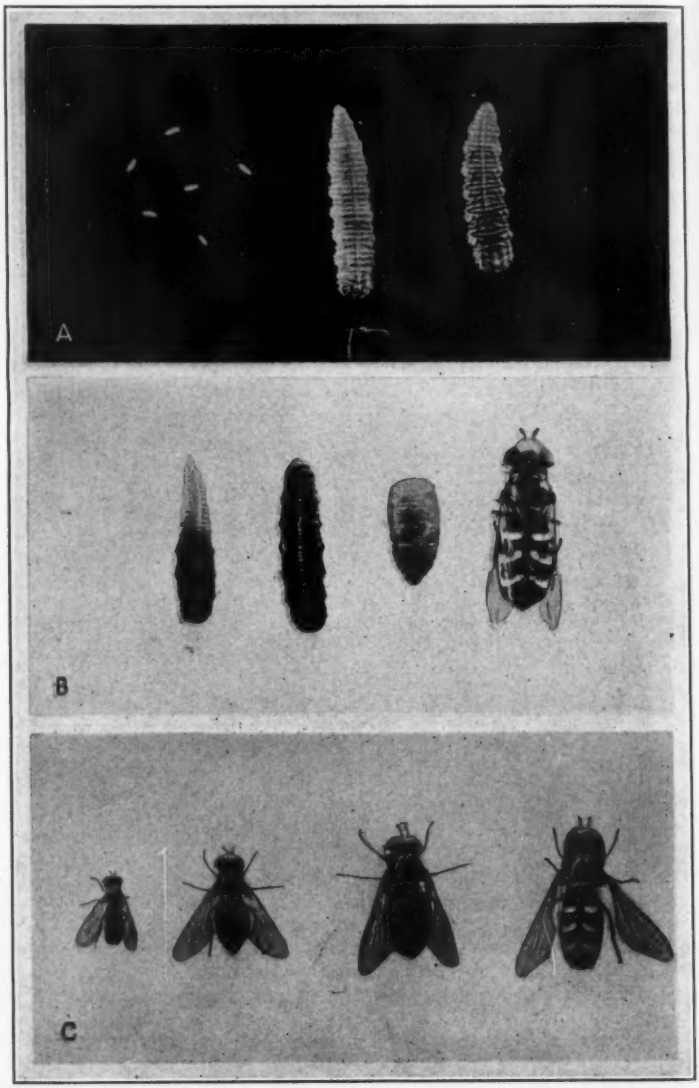

FIG. 7.—Syrphidae predacious on the pea aphis: A, eggs and larvae of *Scaeva pyrastri*; B, larva, pupa, and adult of *C. pyrastri*; C, adults, from left to right, of *Sphaerophoria sulphuripes*, *Eupeodes volucris*, *Syrphus nitens*, and *Scaeva pyrastri*

checking a light infestation of aphids or in preventing a reinfestation after artificial control measures have been adopted.

During a serious infestation the syrphid larvae become very numerous, but by the time they have become abundant enough to be a factor in reducing the number of aphids, the damage to the crop has often been accomplished.

COCCINELLIDAE

Although the lady beetles do not attack the pea aphis as readily as they do some other species, they can always be found in an infested pea field, occasionally in abundance, the larvae and adults feeding greedily.

First in importance of the Coccinellidae found feeding on *Illinoia pisi* is the common *Hippodamia convergens* Guér., and the less common form *H. 5-signata punctulata* Lec. *Coccinella californica* Mann. is also

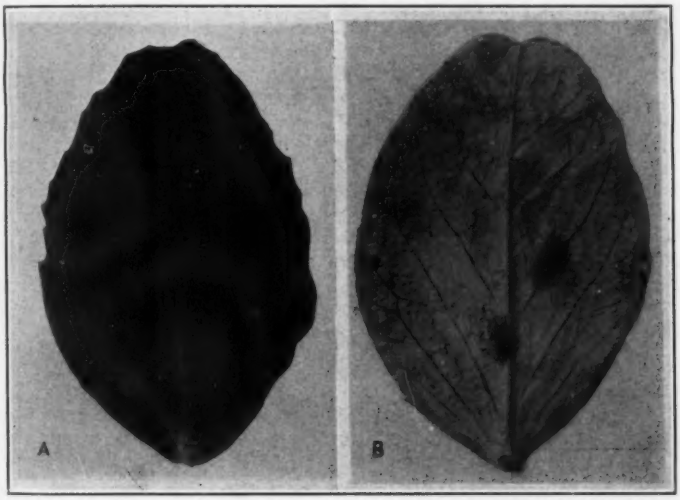

FIG. 8.—A, Parasitized pea aphids, showing mummified bodies; B, aphid killed by fungus compared with healthy specimen

found in considerable numbers, and to a lesser extent, *Cycloneda sanguinea* L., *Olla abdominalis* (Say) and its variety, *plagiata* Casey. Several unreared Scymnus larvae were observed. Two desert species, *Ceratomegilla vittigera* Mann. and *Hippodamia parenthesis* Say, have been found to be quite abundant in several localities of the Mojave Desert, feeding freely on the pea aphis on alfalfa.

The number of pea aphids eaten by the coccinellids varies considerably with the season, weather conditions, and size of the individuals. A larva will destroy from 50 to 350 aphids in from two to four weeks. An adult will consume from 100 to more than 1,000 during its active life, which varies from three weeks to three months. During favorable weather the number of aphids eaten in a day may be as high as 40 to 50 and averages 20, whereas in cold or wet weather the same individuals average only 1 or 2 per day. It is possible for the lady beetles to multiply rather rapidly, for many females lay from 500 to 900 eggs each. These hatch in from 4 to 8 days.

The presence of lady beetles and other natural enemies often keeps an infestation in check and assists in preventing a reinfestation after control measures have been put into operation. Under conditions favorable to the aphids, the lady beetles, as well as the syrphids and other enemies, do not effectively check severe infestations.

PARASITES

Several internal parasites have been observed, but they have never appeared in sufficient numbers to be of material aid in reducing the number of aphids (fig. 8, A). *Praon* sp., *Aphidius rosae* Haliday, *Monoctonus paulensis* Ashm., and *Ephedrus californicus* Baker have been reared from the pea aphis.

FUNGOUS DISEASES

Owing to the low humidity of the California atmosphere, fungous diseases of the pea aphis are usually of little importance. Occasionally, during a rainy period in the spring or fall, or following an irrigation, the common aphis fungus develops, but usually not to a degree sufficient to check infestation materially (fig. 8, B). During the severe infestation of peas in Stanislaus County in 1923, there was a rainy period, after which the aphid fungus developed so rapidly that the infestation was entirely suppressed in about two weeks. At the time of the heavy infestation of alfalfa in southern California in 1924, two rainy periods, together with some irrigation, stimulated the growth of *Entomophthora aphidis* Hoffm. until scarcely a live aphid could be found. *Cladosporium aphids* Thüm has also been observed on occasions to attack the pea aphis.

SUMMARY

The pea aphis is well distributed over California.

It was first reported in California in 1883, and had become widely disseminated by 1910.

Injury is caused by sucking out the plant juices, resulting in weak, stunted plants, misshapen pods, and a material loss in production.

The food plants are largely confined to the pea family, with peas and vetches suffering the greatest injury.

Spreading to some distance is accomplished by winged forms, and from plant to plant by wingless forms.

Serious damage occurs both to the spring and the fall crops.

The life history in California is a continuous series of generations of viviparous females.

The length of the various stages in the life history, and the total number of young of a female vary inversely with the temperature. The number of young produced in a day varies directly with the temperature.

The pea aphis matures on an average in from 7 to 14 days, according to the season. The reproductive period averages from 14 to 17 days for the year.

The yearly average production of young in a day is about 4, and the maximum number produced by one female is 176, the average being 68.

Winged forms require longer to develop and produce fewer young than wingless forms.

Parthenogenetic reproduction continued for 19 months to the forty-fourth generation.

Adverse weather conditions may materially check an infestation of the pea aphis.

The pea aphis is attacked by several parasitic and predacious enemies and by a fungous disease, but these are ordinarily of little importance in checking an infestation.

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BOTRYTIS BLOSSOM BLIGHT AND LEAF SPOT OF GERANIUM, AND ITS RELATION TO THE GRAY MOLD OF HEAD LETTUCE[1]

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INTRODUCTION

In 1916, a species of Botrytis produced a blossom blight and leaf spot on geranium plants (*Pelargonium hortorum*) which were growing in one of the greenhouses of the Kansas State Agricultural College. The disease was first noticed in connection with some hybridization experiments with geraniums. The injury to the flowers was so severe that it was impossible to obtain satisfactory crosses. In 1918, the writer stated in an abstract (*13*) [2] that this disease was causing considerable damage in greenhouses and that the causative organism was closely related to, if not identical with, *Botrytis cinerea* Pers.

On account of more urgent work, no attempt was made at that time to summarize the studies or to continue the experiments already begun. The present paper contains the results of the earlier investigations, together with some additional studies made in 1921–22.

REVIEW OF LITERATURE

No extensive reports can be found in the literature which mention *Botrytis* spp. as causing a blossom or definite leaf-spot disease of Pelargonium. Several magazine articles (*1*, *2*) and reports in floral journals have mentioned "gray mold" or "damping-off" as causing injury to geraniums in the cutting beds and to the leaves of numerous bedding plants, but no reference is made to an injury produced by a *Botrytis* spp. on vigorous flowering geranium plants. This condition, however, has probably been observed by other investigators, although its cause was not known.

Butler (*8*, *p. 66*) mentions *Botrytis cinerea* as attacking geraniums, that foreign matter, such as the petals of the flower, falling onto the leaves, results in leaf infection. In correspondence he states that he was unable to discover the authority from which the assertion in his book is made. Garman (*10*), Chifflot (*9*), and Brierley (*6*) mention that geranium plants are attacked by *Botrytis* spp., infection on the leaves resulting in some instances, but they give no detailed description of the symptoms. A great deal of literature has been published on *B. cinerea* and *B. vulgaris* from the morphological, physiological, and pathological points of view, but these are outside the scope of this paper.

[1] Received for publication Aug. 10, 1925; issued May, 1926. Published, with the approval of the Director, as Paper 246, Department of Botany and Plant Pathology, Kansas Agricultural Experiment Station.

[2] Reference is made by number (italic) to "Literature cited," p. 894.

Mrs. Flora Patterson, formerly in charge of the mycological herbarium, United States Department of Agriculture, furnished the following information, in 1922:

In the majority of instances in citations reporting species of Botrytis on geranium, the parts of the plant attacked are not mentioned. I have compared the macroscopic appearance of the leaves you sent with that of the specimens in the herbarium labeled *Botrytis cinerea or Botrytis vulgaris*, and have found no well-defined differences. I have microscopically compared the fungus on the blossoms sent with the only specimens in the herbarium with *Botrytis* spp. on blossoms and apparently all are the same. The labels on the material here are as follows:

Botrytis vulgaris Fr.:

On Pelargonium (cult. geranium) blossoms and leaves. Dept. Agr. Grounds, Washington, D. C. F. W. Anderson, Sept. 5, 1890. Det. F. W. A.?

Botrytis vulgaris Fr.:

On Pelargonium Sp. (blossoms). College Park, Md. Oct. 30, 1908. Comm. A. J. Norman. Det. F. W. Patterson.

In our subject catalogue there is a note saying that specimens were brought to this office by Doctor Hasselbring June 29, 1915, that were collected in a greenhouse in Michigan, where over 1,000 plants were dwarfed and the leaves had turned yellow. My note states that Botrytis was found on the flowers.

The office of plant disease records, United States Department of Agriculture, provided the following information from reports which appear to be the only records in this country concerning the occurrence of *Botrytis* spp. on geranium plants:

SURVEY NOTES

1908. Reported from Baltimore and Prince Georges Counties, Md., by J. B. S. Norton. Type of injury not stated.
1909. Reported from College Park, Md., by J. B. S. Norton. He mentions it as a leaf blight.
1913. Reported from Michigan by G. H. Coons. Single instance in greenhouse in Joseph County. Common name, gray mold. Type of injury not stated.
1920. Reported from Riley and Linn Counties, Kans., by L. E. Melchers. Attacks foliage and flowers.
Reported from Madison County, Ohio, by A. D. Selby. Calls it *Botrytis cinerea*. Type of injury not stated.
1921. Reported from Kansas by L. E. Melchers. Quite serious during past seasons.
1922. Reported from Rooks County, Kans., by L. E. Melchers.

SPECIMENS IN PATHOLOGICAL COLLECTIONS, UNITED STATES DEPARTMENT OF AGRICULTURE

Botrytis vulgaris Fr.:

(1) On blossoms, collected at College Park, Md., October 30, 1908. A. J. Norman.
(2) On leaves, Washington, D. C., collected by D. G. Fairchild, March 10, 1890. "Seems to be doing some damage in greenhouses about here."

Botrytis cinerea Pers.

(1) On leaves and stems on geraniums potted and in cold frames. Terminal leaves curl and are partially aborted. L. Foster. New Orleans, La., 1919. First noted last year in a shipment of some plants of "Jean Viaud" variety from Macgregor nurseries, Cleveland, Ohio. Abstract from letter with specimen. Specimen showed leaves.
(2) On leaves collected in Konigstein (probably Germany) by W. Krieger.

ECONOMIC IMPORTANCE

Since working with the Botrytis blossom blight and leaf spot of geranium the writer is convinced that very few persons have associated the "early fading" or drying up of geranium blossoms with this fungus. Such a condition has most frequently been assigned to

"natural causes." This disease is slightly less common on plants that are growing rapidly, although it should not be understood that it attacks only weakened plants. The blight of the blossoms and the leaf spots may occur abundantly on the average commercial greenhouse geranium plants, for it seems that the environmental conditions are more important in determining the presence of the disease than the age or vigor of the host. The conspicuous brown spotting of the foliage produces unsightly plants and reduces the vitality of the plants materially. The blossoms, particularly the double ones, frequently "fade" or dry a week or 10 days sooner than they should, which naturally makes the plants less desirable. A geranium plant can not be readily sold by the florist unless it has at least one satisfactory flower.

It was noticed during these investigations that in adjoining greenhouses where head lettuce was grown serious outbreaks of gray mold were present. This led the writer to wonder whether the species of Botrytis occurring on geraniums could be responsible for the disease on head lettuce, or vice versa. The symptoms of gray mold are well known to plant pathologists, as well as the damage that it may cause to the head-lettuce crop in the greenhouse. These questions, in addition to morphologic and physiologic studies of the organism, are fully discussed in the literature (*7; 3; 4, p. 219, 224, 380; 11; 12; 14; 15; 16; 17.*) Gray mold has ruined several crops of head lettuce in the greenhouses at the Kansas State Agricultural College.

In the discussion that follows it will be shown that the species of Botrytis causing blossom blight and leaf spot of geraniums may be responsible for the gray mold of head lettuce, and vice versa, so the disease on one of these crops may be responsible for the appearance of the disease on the other.

SCOPE OF THE INVESTIGATIONS

These investigations cover the following phases: (1) Symptoms of the blossom blight and leaf spot of geranium; (2) the isolation of the causal organism of the geranium blossom blight and leaf spot, with pathogenicity studies; (3) brief studies of the cultural and microscopic characteristics of the organism; (4) the relation of the geranium blossom blight and leaf spot to the gray mold of head lettuce; and (5) control measures.

SYMPTOMS OF THE BLOSSOM BLIGHT AND LEAF SPOT OF GERANIUM

The blossom blight of geraniums produces the premature fading and drying of the petals of the flowers. This disease is more severe on the double than on the single varieties, this being due no doubt to the larger number of petals in a floret tending to retain moisture. The petals first begin to turn dark at the margins and wilt as though they were fading from age. Generally the central florets are the first to show infection. In red-flowered geraniums, infected petals turn very dark (pl. 1). This generally occurs several days in advance of the time when they should naturally fall. If sufficient moisture collects on the blossoms from syringing, watering the plants, or from leaky glass roofs, the fungus will sporulate abundantly on the infected tissue. Fully developed blossoms show the symptoms most strikingly, but very frequently young blossoms will show floral infection, even before all the buds are open (pl. 1, C, D, E).

For a time the writer was at a loss to understand what was causing the more or less large, somewhat circular, spots on the leaves. It finally was discovered to be due to such material as falling petals which were infected with *Botrytis* spp. These readily infect the leaves, the infected areas assuming the outline of the infected material that happens to fall thereon (pls. 1 and 3, A, B). Sometimes the margins of the spot conform very definitely to the shape of petals which fall upon the leaf. If, however, sufficient moisture is present, the spots enlarge, become irregular in outline, and from one-fourth to practically the entire leaf, including the petiole, becomes infected (pl. 3, A). If the conditions are less favorable for the spread of the fungus, only a definite spot results. When the lowermost leaves become contaminated by falling petals, the entire leaf generally becomes infected, since less circulation of air and sunlight strikes such leaves and the relative humidity is greater. This condition is especially noticeable when plants are somewhat crowded on the greenhouse bench. Sometimes a marginal infection of the leaves results, with smaller spots on various parts of the leaves. The marginal infection is less common and seems to be the result of direct infection from the fungus, being carried from the soil and other sources by air currents rather than a direct contact infection from diseased petals. It has been generally observed in such a case that the fungus begins to develop but is inhibited in its spread so that large areas are not involved.

Any portion of the geranium plant having become infected may give rise to the conidia and conidiophores in a few days, if placed in a damp chamber. A great abundance of conidiophores produced on the leaves is shown in Plate 3, A, B. Masses of conidia may be produced in the greenhouse on affected tissues if sufficient humidity is present and the sunlight is not too intense. This is especially evident on infected leaves that are somewhat shaded.

ISOLATION AND INFECTION EXPERIMENTS

It was a simple matter to isolate the organism by first carefully sterilizing infected petals, small fragments of leaf spots, or petioles, with a 1 : 1,000 solution of mercuric chloride, carefully washing in sterile distilled water, and plating on potato agar. Within 48 hours at 22° C. a distinct mycelial growth occurred. No other organism appeared in the Petri dishes. In order to obtain pure line or strain cultures for experimental work, conidia were plated and cultures transferred from well-isolated colonies. These were used for artificial inoculations, being grown on Thaxter's hard potato agar and on carrot agar.

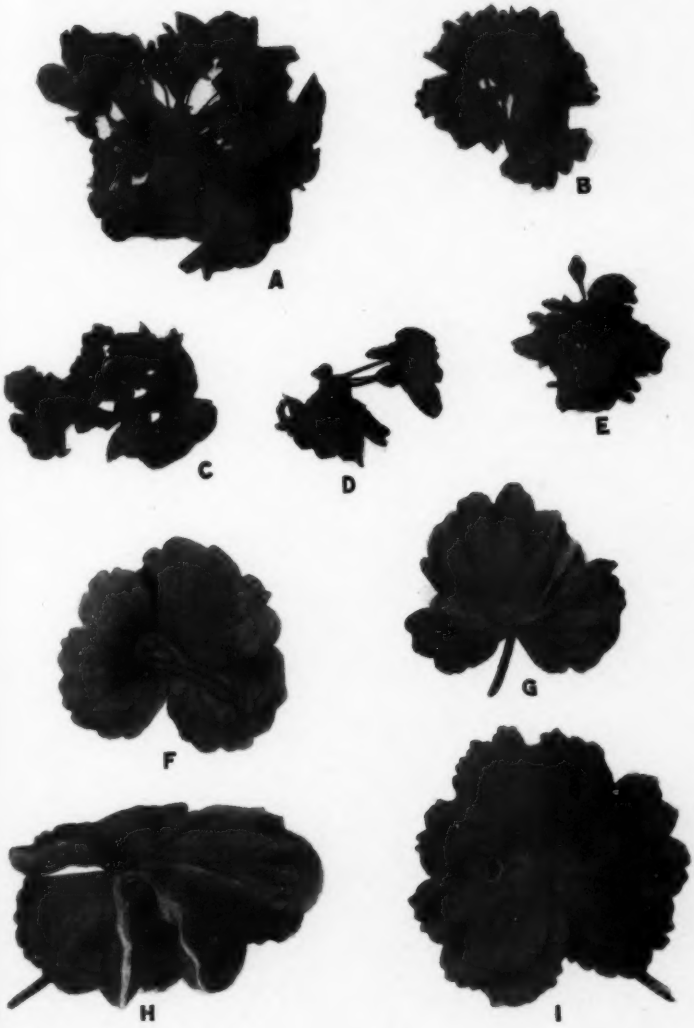
A large number of potted geranium plants in various stages of flower development were obtained for inoculation. Known varieties were used where possible, but in some instances this was not feasible.

EXPLANATORY LEGEND FOR PLATE 1

Botrytis blossom blight and leaf spot of geranium. The central florets are attacked first. The petals of red varieties turn dark

A to I, inclusive.—Various stages of development of infected flowers, together with several leaf spots; the result of artificial inoculation. The flowers were sprayed with a decoction of Botrytis conidia, while the leaf spots resulted from natural inoculum (infected petals) falling on the leaf. Very young as well as fully-developed flowers and leaves may be attacked

F, G, H, I.—Leaf spots resulting from fragments of infected geranium material coming in contact with geranium leaves. F and G, the same leaf at different stages of infection. F, the conidiophores sporulating


A
B
C
D
E
F
G
H
I

Some of the plants were inoculated and placed under bell jars which were whitewashed or covered with newspapers. Other plants were inoculated and placed on the greenhouse bench without bell-jar covering.

The results of a set of inoculations where the plants had been covered with bell jars are recorded in Table I. In this experiment geranium plants were obtained from another greenhouse in Manhattan where there were no signs of the disease. Twelve plants of various varieties having from one to three blooms of the double-petal type were used for the first set of inoculations. A suspension of conidia was made by gently shaking and stirring sterile distilled water with cultures of Botrytis. Fragments of conidiophores and mycelium probably also were removed, since no special attempt was made to separate the spores from mycelial fragments. This suspension was atomized onto the blossoms of the geraniums by means of a DeVilbiss atomizer. The tissue of the host was not punctured or mutilated in any manner, and no wounds were noted at time of inoculation. Sterilized newspapers were placed over the sand on the greenhouse benches and the potted plants set on the newspapers. Sterile whitewashed bell jars were placed over the inoculated plants. The temperatures ranged from 60° F. at night to 90° F. during the daytime. Control plants were atomized with distilled water and placed under the bell jars in a similar manner.

TABLE I.—*Geranium plants artificially inoculated with Botrytis spp., and placed under bell jars in the greenhouse*

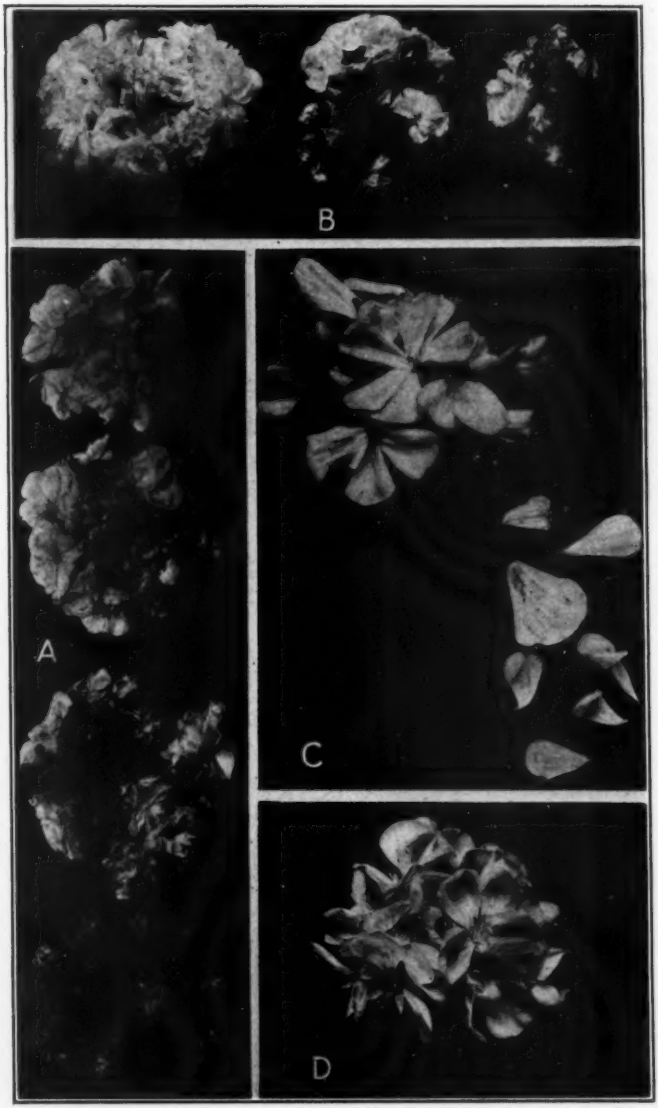
Number of plants inoculated	Plants infected		Remarks
	Number	*Per cent*	
*12	12	100	First visible signs of petal infection were noted three days after inoculation. At the end of five days all plants showed definite signs of infection.
3 (controls)	0	0	

* Names of varieties in this series not definitely known.

At the end of five days the conidia were appearing on the petals of some of the central florets of several of the flowers. In seven days the peduncles were blackened and giving rise to conidiophores (pl. 2, A). A large number of leaf spots occurred where falling petals came to rest upon leaves, these gradually involving the entire leaves and petioles. Plate 3, B, shows infections as they appeared at the end of three days.

No infection occurred on the flowers of the control plants although at the end of eight days a normal petal fall occurred.

A second set of experiments was planned in which the inoculated plants were not placed under bell jars. The same method of inoculation was used, and the plants were placed in an adjoining greenhouse used by the department of agronomy. No geranium plants or other varieties of bedding plants known to be affected by *Botrytis* spp. were grown in this greenhouse. The conditions were less favorable for infection than is generally the case in greenhouses where bedding plants are grown, as there was a very much lower relative



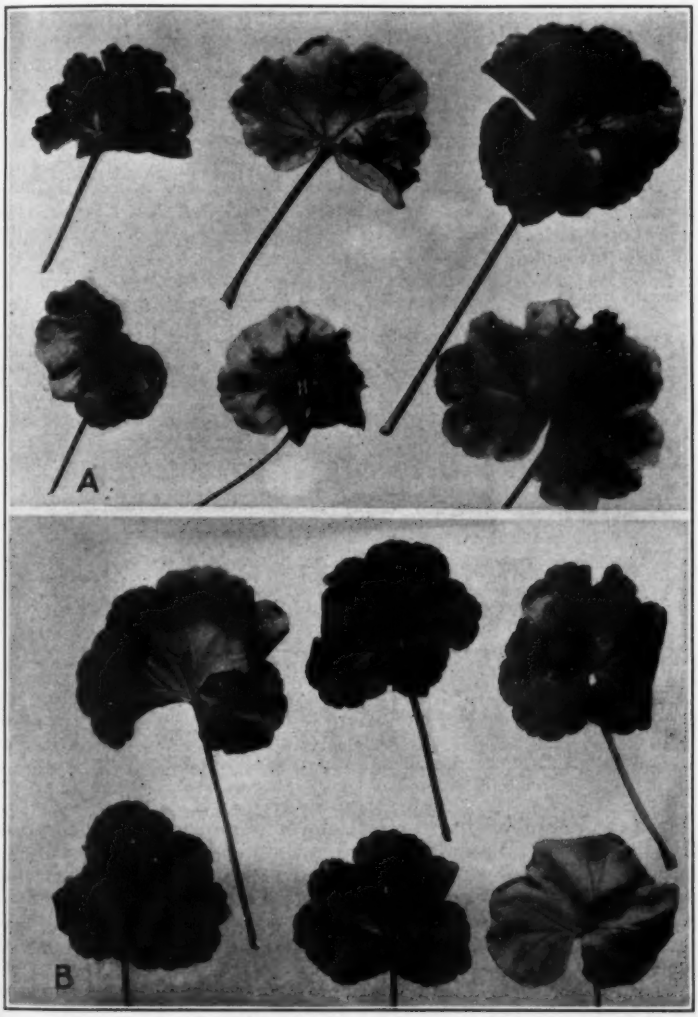
Various stages of geranium Botrytis blossom-blight flower infection

A.—Several flowers showing various degrees of infection. Flowers atomized with a suspension of conidia in sterile distilled water. Infection became visible at the end of 3 days. Plants under bell jars

B.—Same as A, except plants were not under bell jars

C.—Control plant for series of inoculations under bell jars shown in A Note normal peta fall, and no sign of "fading" or drying of petals

D.—Control plant of series of inoculations in B, and not under bell jar



Various stages of geranium leaf-spot infections which occur naturally and which have been produced by artificial inoculation

A.—Natural leaf infection by *Botrytis* spp., as collected in the greenhouse
B.—Leaf spots, following placing of infected petals on leaves

humidity. Even under these conditions infection occurred, but the fungus did not appear superficially as in the experiment under bell jars. The results of these inoculations are given in Table II, and the degree of infection is illustrated in Plate 2, B.

TABLE II.—*Geranium plants artificially inoculated with Botrytis sp., and not placed under bell jars*

Variety	Blossoms on plants	Stage of bloom	Date of inoculation (1917)	Date of first signs of infection (1917)	Final note on infection	Remarks
Jean Viaud	[a] 2	Part of florets in full bloom.	[b]		No infection	Atomized with distilled sterile water and placed on greenhouse bench.
La Favorite	[a] 2	One in bud stage; one in full bloom.	[b]		do	Do.
La Favorite (No. 1).	1	Full bloom	May 1	May 3	Petals of all the florets in the center of the flower were brown and dry.	Four days after inoculation the fungus showed a slight superficial growth.
La Favorite (No. 2).	3	Full bloom, semibud, and bud.	do	do	Florets on all three flowers showed distinct infection.	
La Favorite (No. 3).	1	Full bloom	do	do	Only one floret in the entire flower seemed to escape infection.	
La Favorite (No. 4).	4	2 full blooms; 2 half blooms.	do	do	From one-half to the entire flower infected in all 4 flowers.	
Peter Henderson (No. 5).	1	Full bloom	do	do	4 florets infected, dry, and "faded."	
Jean Viaud (No. 7).	1	Semibud	do	do	6 florets infected, dry and brown.	
Jean Viaud (No. 7).	1	One-quarter full bloom.	do	do	1 floret distinctly infected, with few surrounding petal infections.	
Jean Viaud (No. 8).	1	Bud	do	do	1 floret slightly infected in the center of the flower.	
Jean Viaud (No. 9).	1	One-quarter full bloom.	do		No apparent infection.	
Jean Viaud (No. 10).	1	Half, full bloom.	do	May 3	3 florets distinctly infected, dry and "faded."	

[a] Control. [b] Not inoculated.

It was a simple matter to reisolate the organism from the various plant tissues which became infected in these inoculation experiments. Conditions were not sufficiently humid in the greenhouse to obtain leaf spot infections from falling petals. Later several of the plants were removed to humid conditions in another greenhouse and without the aid of bell jars, typical leaf spot infections were obtained from infected petals.

Several other series of inoculations were conducted, and the same results were obtained. One difficulty encountered was the obtaining of a sufficient number of plants that were absolutely free from Botrytis infection before the experiment was begun. In one instance two of the six controls showed signs of infection, and the entire experiment had to be abandoned.

CULTURAL AND MICROSCOPIC CHARACTERISTICS OF THE ORGANISM

CULTURAL CHARACTERISTICS

The various cultures obtained from tissues of petals, petioles, and leaf spots of the geraniums as they were growing in the greenhouse apparently were the same species of Botrytis. At least no essential or striking differences in growth or color could be observed on culture media; macroscopically, at least, the various cultures resembled each other. They grew vigorously on congenial media, producing at first a pale or light gray color, followed by a gray to gray-brown hue as sporulation began. Conidia were produced abundantly in rather loose masses on the branched conidiophores. Sclerotia were also abundantly produced as the cultures aged. Growth habits indicated that it is not one of the degenerate types of Botrytis. Very marked differences, however, were observed in the rate of growth, sporulation, and sclerotial production when the organism was grown on several different media. The cultures were not grown at various temperatures. The observations were made, and the data in Table III were taken when the strains [3] were grown at 21° to 22° C.

TABLE III.—*Various media used for growing Botrytis spp., arranged in the order of their importance for the best growth of the organism*

[Notes were taken at the end of 30 days' incubation at 21° to 22° C.]

Medium	Vigor of growth	Sclerotial production
Potato plugs	Very vigorous, first to sporulate	First to produce sclerotia in abundance, which were the largest produced in any medium (10 mm.).
Potato hard agar	Very vigorous	Sclerotia in abundance.
Carrot agar	do	Do.
Glucose agar	do	Do.
Pepper (sweet) agar	do	Do.
Steamed rice	Slow to start, moderately vigorous later	Weak production of very small sclerotia.
Bean agar	Vigorous	Sclerotia moderately abundant.
Sorghum agar	do	Sclerotia abundant.
Prune agar	Moderately vigorous	Do.
Plain	Moderately vigorous, very slight sporulation	Sclerotia moderately abundant.
Oatmeal agar	Moderately vigorous, no sporulation	Sclerotia abundant.
Corn-meal agar	Very weak, practically no growth	No sclerotia.

MICROSCOPIC CHARACTERISTICS

The several strains which were isolated, besides appearing the same macroscopically, were as far as the writer could determine, microscopically similar. As is the case with several other genera of fungi, the genus Botrytis is a difficult one to deal with taxonomically. In view of the existing confusion it seems best to consider the organism under investigation as being a Botrytis species belonging to the cinerea type. Cultures were submitted in 1917 and 1922 to H. H. Whetzel and Mrs. Flora Patterson, respectively. In their opinions the cultures belonged to the cinerea group.

The conidia averaged 9.3 μ by 13 μ in size, and ranged from 8.3 μ to 10.6 μ in width, and from 11.2 μ to 14.5 μ in length. The measurements were taken from spores of cultures grown on carrot agar.

[3] The use of the word "strain" has reference to the various single-spore cultures which were made.

These measurements are within the range of the species cinerea. The spores germinated very readily in sterile water or carrot decoction, and produced germ tubes within 24 to 30 hours at 22° C. The spores likewise germinated very readily on nutrient agars. The rate at which the germ tubes develop and the mycelium grows and produces conidiophores depends largely on the medium and temperature.

RELATION OF BLOSSOM BLIGHT OF PELARGONIUM TO THE GRAY MOLD DISEASE OF HEAD LETTUCE

Since these strains of Botrytis resembled so closely the organism known to be the cause of the gray mold of head lettuce (*Botrytis cinerea* Pers.), it was of interest to try to discover whether the strains from the geranium were capable of producing the typical gray mold disease of head lettuce.[4] Several varieties of head lettuce—including Mignonette, Crisp Head, Henderson's Unrivaled, Denver Morehead, and California Cream Butter—were grown to the "heading stage," at which time gray mold is known to attack head lettuce, and a conidial suspension in sterile distilled water, made as already described, was atomized onto the plants. The lettuce plants were growing in soil on a greenhouse bench. The plants inoculated were inclosed by panes of glass so as to make a compartment over which newspapers were laid. Parts of each row of the several varieties were used as controls. These plants were atomized with sterile distilled water, inclosed in a glass compartment, and covered by newspapers in a manner similar to that of the inoculated plants. No punctures or other mechanical injuries were made in the inoculation work. Tipburn on the leaves was not observed. The first evidence of infection was observed within five days after inoculation, and at the end of seven days vigorus infection was in evidence on all varieties. The controls were entirely free of any signs of gray mold. Isolations were made from the tissue of various heads. A strain of Botrytis which was recovered was again used to inoculate geranium plants, with positive results in every instance. There is indication, therefore, that the Botrytis species causing the blossom blight of geranium is capable of producing the gray mold of head lettuce.

About a year after these experiments were conducted, a crop of head lettuce growing in the horticultural greenhouses was severely attacked by gray mold. Since this was a natural occurrence of the disease, it afforded an opportunity to make some cross-inoculations with geraniums. Isolations were made from the head lettuce and grown on potato agar. When conidial production occurred, a sterile distilled-water suspension was made of the conidia and atomized onto the blossoms of geranium plants in various stages of bloom. Within a few days the typical blossom blight occurred. This proved definitely that the species of Botrytis capable of causing gray mold of head lettuce is also pathogenic on the common geranium, where it may cause a definite blossom blight and leaf spot.

Observations frequently made by persons acquainted with greenhouse conditions show that a species of Botrytis (considered to be *B. cinerea* Pers.) occurs on organic matter in and on the sand of cutting benches, moist soils, and elsewhere. This no doubt serves

[4] John T. Pearson, a student in the department, aided in these studies.

as a source of inoculum. Under favorable environmental conditions and the presence of susceptible hosts *Botrytis cinerea* is parasitic and may cause considerable damage.

Conditions which favor its occurrence and spread are an abundance of moisture and a lack of sufficient ventilation. Plants that are crowded together will retain moisture for periods sufficiently long to make conditions favorable for infection. The sand of cutting benches is especially likely to harbor the spores of Botrytis.

SUGGESTIONS FOR CONTROL

Bewley (*5*), in a discussion of diseases of greenhouse plants, mentions the importance of Botrytis, gives a list of crops attacked, and suggests methods for control. Essentially, the methods advocated are those which pertain to sanitation.

The blossom blight and leaf-spot disease of the common geranium is one which can be largely controlled by sanitary measures in the greenhouse. Sources of inoculum must be discovered and removed. Disinfecting the cutting beds and using new soil which has been sterilized may help. Care in watering and syringing the plants is essential in checking the disease. This must be accompanied by proper and sufficient ventilation, so that the leaves and flowers will not remain moist for long periods.

Geranium plants should be separated sufficiently on the bench to allow proper air circulation and sufficient sunlight. The prompt removal and destruction of all affected parts is advised. Débris should not be thrown on the floors or be allowed to remain on the benches.

SUMMARY

A species of Botrytis which in all essentials is of the cinerea type may cause a blossom blight and leaf spot of the common geranium (*Pelargonium hortorum*).

Infected petals or other plant fragments falling onto the leaves of geraniums produce definite leaf spots or completely destroy the leaves.

This species of Botrytis may cause the gray-mold disease of head lettuce in the greenhouse.

The species of Botrytis causing the gray mold of head lettuce (*Botrytis cinerea* Pers.), when it occurs naturally in the greenhouse, may produce the blossom blight and leaf spot of geranium.

Macroscopically and microscopically, the species of Botrytis causing the geranium disease resembles *Botrytis cinerea* Pers., there being no evident essential difference.

The early "fading" and drying of the blossoms and the unsightly leaf spots reduce the value of the geranium plant for ornamental purposes.

Sanitary measures in the greenhouse, including proper watering and ventilation, together with care in avoiding the accumulation of refuse, will aid in holding this disease in check. If geranium plants are given sufficient room on the bench so as to admit an abundance of sunlight and proper air circulation, this disease is less prevalent.

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